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distribution unlimited**12b. DISTRIBUTION CODE****13. ABSTRACT (Maximum 200 Words)**

The goals of the **Advanced Cancer Detection Center** of the H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. In addition, the Center created and supports education programs to provide increased cancer awareness and established working collaborations with the James A. Haley VA Medical Center, the Bay Pines VA Medical Center, and the MacDill Air Force Base Hospital. The projects included in this report are:

- Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (PI: Tockman)
- Breast Cancer Early Detection Study: Feasibility of Detecting and Characterizing Molecular and Antigenic Markers in Fluid from Breast Nipple Aspirate (PI: Shaw)
- Genetic Analysis of Familial Prostate Cancer (PI: Sutphen)
- The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (PI: Kumar)
- Specific Role of Genistein and Breast Cancer Risk (PI: Kumar)
- Phase IIA Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer (PI: Shaw)
- Development of the Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers (PI: Krischer)

Each of these projects is presented as a complete study in the attached materials.

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Table of Contents

	Page
Report Documentation Page.....	2
Forword.....	3
Table of Contents	4
Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (DOD Cohort Study) Principal Investigator: Melvyn Tockman, M.D., Ph.D.	5
Breast Cancer Early Detection Study: Feasibility of Detecting and Characterizing Molecular and Antigenic Markers in Fluid From Breast Nipple Aspirate Principal Investigator: Gail L. Shaw, M.D.....	16
Linkage Analysis of Hereditary Prostate Cancer Families Principal Investigator: Rebecca Sutphen, M.D.	31
The Specific Role of Genistein in Estrogen Metabolism Principal Investigator: Nagi Kumar, Ph.D., R.D.....	33
The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer Principal Investigator: Nagi Kumar, Ph.D., R.D.....	39
Phase IIa Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer Principal Investigator: Gail L. Shaw, M.D.....	44
Development of the Moffitt Cancer Network as a Telemedicine and a Teleconferencing Educational Tool for Health Care Providers Principal Investigator: Jeffrey P. Krischer, Ph.D.	59

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (DOD Cohort Study)

Principal Investigator: Melvyn Tockman, M.D., Ph.D.

Introduction

Two promising and practical screening techniques, computerized molecular analysis of airway cell markers (ACM) and helical computed tomography (CT), are now available to examine targeted populations for the earliest signs of lung cancer. Henschke et al. (*Lancet*, 1999 354:99-105) found that 10% of helical CT-detected noncalcified nodules from 2-5 mm through 21-45 mm contained a primary lung cancer. This is four times the sensitivity of a standard chest x-ray taken at the same time. Our preliminary data (*Clin Cancer Res*, 1997, 3:2237-46) showed that computerized immuno-detection of up-regulated hnRNP A2/B1 expression in sputum cells detected primary lung cancer in 37 of 45 (82%) cases. This is 8 times the sensitivity of standard sputum cytology obtained at the same time.

It is quite likely that the helical CT and protein expression screening of ACM are complementary. The cell type distribution of the detected cancers suggests this. Henschke reported that of the 27 tumors identified by helical CT, 21 (78%) were adenocarcinomas (includes 3 bronchioalveolar carcinomas), 3 (11%) were mixed squamous-adenocarcinoma, 1 (4%) was a squamous and 1 (4%) was an atypical carcinoid. In contrast, among the 45 primary lung cancers detected by ACM protein expression screening of Yunnan tin miners, we found 4% were adenocarcinoma, 51% were squamous, 2% were large cell, and 2% other (40% of these cases lacked a histological diagnosis). Of the 13 second primary lung cancers detected by protein expression screening, 31 % were adenocarcinoma, 23% were squamous, 15% were mixed adenosquamous, 15% were small cell, 8% were large cell and 8% non-lung primary. Evaluation of the extent to which these early lung cancer detection techniques are complementary could only be conducted in a one-arm prospective study such as this one, where every individual is screened by all techniques at the same examination.

Several years ago with David Sidransky at Hopkins, we pioneered the use of microsatellite alterations as clonal markers in the detection of human cancer (*Proc Natl Acad Sci USA* 1994; 91:9871-75). We have found that microsatellite alteration and LOH on 3p is significantly associated with upregulation of hnRNP A2/B1 (*Proc. AACR* 1999; 40:140-1). Further, loss at 3p22, the site of gene for the Type II Transforming Growth Factor Beta Receptor is strongly associated with NSCLC. Alteration of the tumor suppressor TGF- β signaling pathway is of great interest in our laboratory. Therefore, we have developed a technique for preservation of sputum morphology and nucleic acids so that (DNA) microsatellite alterations as well as altered TGF- β type II receptor message expression (RNA markers) may be examined in the sputum specimens collected in this study.

The populations of greatest interest for lung cancer screening are the estimated 46 million former smokers in the United States who remain at risk although they have stopped smoking. While cardiovascular risk resolves on smoking cessation, genetic alteration of airway lining cells observed in current smokers is not reversed in former smokers (Gazdar et al.). Progression to

lung cancer is probably only slowed by removal of the promotional stimuli of smoking. Major medical centers (Beth Israel, M. D. Anderson) now report more new cases of lung cancer from former than from current smokers.

Age and cigarette smoking are not the only risk factors. We have shown that current and former smokers with airways obstruction are at 2-4 fold risk of developing lung cancer compared to non-obstructed smokers (*Ann Int Med* 1987;106:512-8). A population of obstructed current and former smokers is identified and available through the Respiratory Division of the James A. Haley VA Hospital. This population has been selected to initiate the present study comparing the accuracy (sensitivity and specificity) and predictive value for detecting pre-clinical lung cancer by ACM of upregulated gene expression of hnRNP A2/B1 and by helical CT scanning.

Body

Start-up: Study Activation, 0-3 Months

Approval: The protocol, informed consent and data collection forms were completed and this study was approved by Moffitt/USF IRB on November 5, 1998, with conditional approval by Army Regulatory Compliance on December 23, 1998. The protocol was resubmitted with amendments covering novel methods of sputum preservation to the Moffitt/USF IRB and received Army Regulatory Compliance final approval and study activation on June 10, 1999.

Staff Hired: A total of 6.5 FTE's are now working on this project; 2/3 of these (4.3 FTE) were newly hired to work directly on participant accrual, registration, interview and specimen collection. This distribution of personnel reflects the importance of patient accrual and specimen collection to the success of this study.

Space Renovation: Two spirometry/sputum induction facilities have been established. One at the Lifetime Cancer Screening Center is fully operational. This facility includes a spirometry screening station, a laminar-flow sputum induction hood, and a biosafety cabinet for sputum specimen processing. Interviews and blood drawing also take place in this space. The screening station at the James A. Haley VA Hospital is established but not yet in use due to minor equipment needs. The patient accrual staff have WOC privileges at the VA Hospital.

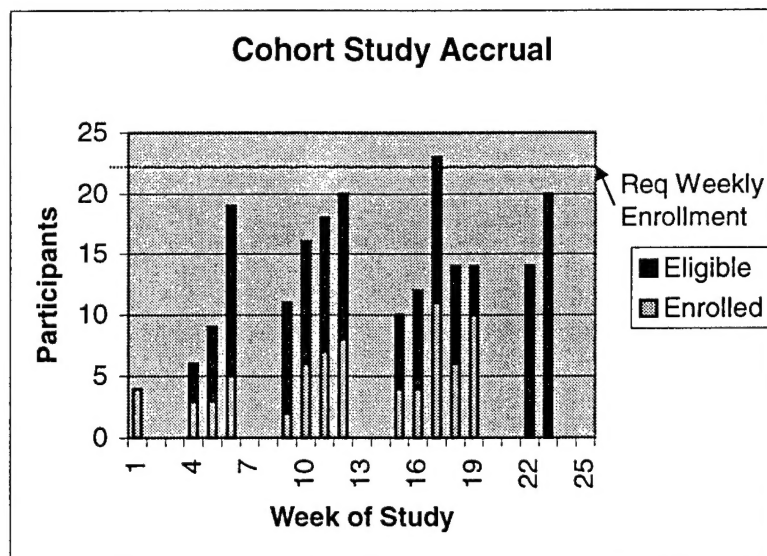
Equipment Purchased: Several major pieces of equipment have been purchased to support this study. These include a Helical CT scanner, a Perkin-Elmer 310 gene scanner, and an Arcturus PixCell II Laser Capture Microdissection device.

Initial Recruitment Begins, 3-15 Months

Accrual: The study plan provides that during the first year, 5,000 subjects ≥ 45 years of age with ≥ 30 pack years of smoking will be screened by spirometry to identify 1,150 subjects with mild obstruction ($FEV_1/FVC \leq 70\%$). Mild obstruction would be expected to occur in 23% of these individuals. Our prior sputum/CXR screening trials have shown that in males of this age range with this smoking history, clinical lung cancer will have a 0.7% (7/1,000) prevalence and 0.5% (5/1,000) annual incidence. In the presence of mild obstruction, the annual lung cancer incidence

increases to 1.1% (11/1,000) and continues to rise with increasing obstruction. After four years of screening and depending upon the prevalence of obstruction in the study population, therefore, we would expect 44-50 cases of lung cancer (11-13 cases per year).

At this time, 176 subjects have been screened with spirometry. Of these, 109 (62%) met obstructive criteria and have proceeded on to sputum induction and helical CT. Thirty-eight (35%) of the screening helical CT scans have shown an abnormality (non-calcified nodule). Of 24 individuals who have gone on to diagnostic CT scanning, 18 (75%) have shown abnormalities (14 lesions of 1-5 mm diameter; 11 lesions 6-10 mm diameter).



Archive: One hundred and seven sputum specimens have been prepared with dithiothreitol (DTT) and EDTA, washed in Hanks solution, spun, resuspended and divided into aliquots for pap staining, immunostaining and storage on slides, in alcohol slurry and freezing with DMSO in liquid nitrogen. From a similar number of blood specimens, the buffy coats have been separated and stored in liquid nitrogen. Each specimen is bar coded, and computer linked to the database of registration, demographic, medical, smoking, occupational and nutritional history data on each participant.

Database and Lab Specimen Tracking System: Moffitt Cancer Control Research Computing has developed an Oracle database with a Web front-end to allow registration from multiple sites. This database houses the registration, demographic, medical, smoking, occupational and nutritional history data on each participant. Since data entry is still forms-based, the data system was designed to provide easy, intelligent 'double' entry of data. The system has been programmed to provide data constraints, range and referential checks, and edit capability to keep the data clean. The data system provides tools for subject management (generate barcode labels, track unresolved data, report late forms/specimens, etc.). Finally, the relational database will easily provide data for specific queries and statistical analysis.

Moffitt Cancer Control Research Computing also has developed a Laboratory Specimen Tracking System. This study generates a large number of specimens that must undergo multiple assays in several laboratories. The Laboratory Specimen Tracking System (LST) will read the 2-D specimen barcode to log the specimen into the laboratory. The LST has been programmed to assign each type of specimen a 'profile' that specifies what will happen to the specimen in the lab. A 'profile' consists of a number of steps such as: Check In/Check Out, Assay specimen acceptability, Results Reporting and Archive. The LST will be able to track the progress of the specimen and let the lab manager know what step the specimen is on, the specimen turnaround time in the lab, and the archive location of the specimen and its offspring including: Slides, Sputum Slurry Bottles, and Cryovials.

Key Research Accomplishments

- Developed an infrastructure to identify, accrue, screen and follow a non-diseased community-dwelling population at high risk for lung cancer.
- Developed procedures for collection and preservation of sputum specimens for new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.
- Developed an archive of airways cytologic specimens suitable for evaluation of new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.
- Developed an archive of white blood cells suitable to provide individual control specimens for DNA and RNA.
- Developed a potency assay for MoAb 703D4 immunodetection of hnRNP A2/B1 protein expression.

Reportable Outcomes

Presentations Related to this Study

- | | |
|---------------------|--|
| December 8-10, 1998 | <i>International Conference on Prevention and Early Diagnosis of Lung Cancer, Johns Hopkins Lung Project and Immunocytochemical Screening for Lung Cancer.</i> University of Varese and University of Massachusetts Medical School, Varese, Italy. |
| February 12, 1999 | <i>ALCASE Workshop – Lung Cancer: A Revolution in Care, Technology in Early Diagnosis of Lung Cancer.</i> Embassy Suites, Tampa, Florida |
| April 26, 1999 | <i>1999 ALA/ATS International Conference Program, Early Sputum Marker for Lung Cancer (hnRNP).</i> San Diego Convention Center, San Diego, California |
| April 30, 1999 | <i>Pharmacology Seminar Program, Detection and Immunostaining of the Lung Cancer Sentinel Cell.</i> University of Pittsburgh, Pennsylvania. |

September 13, 1999 *Advanced Cancer Detection Center, External Advisory Committee.* H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida

September 30 to
October 3, 1999 *The First International Conference On Screening for Lung Cancer,*
Cornell University, New York

The first International Conference on Lung Cancer Screening was held in October 1999. A group of international experts in imaging, molecular diagnostics, pulmonology, oncology, epidemiology, clinical trial design, statistics, health care policy and patient advocacy met to address the issues central to lung cancer screening. The meeting was co-sponsored by the ACS, the National Cancer Institute, ALCASE, Weill Medical College of Cornell University and other organizations. The conference reviewed currently available data on lung cancer screening and engaged in intensive analyses of the implications with a view to attaining consensus with respect to the main issues surrounding early detection of lung cancer. The conference organizers asked for two reports of this study "Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers." Dr. Robert Clark, Moffitt Director of Radiology described lung cancer screening with helical CT, while Dr. Tockman presented the Moffitt experience with protein expression screening of ACM.

The Moffitt trial comparing ACM and helical CT was recognized as one of three such studies in the nation that was capable of providing insight into definitive clinical trial design considerations. ("The sites at which CT screening is currently being performed in the United States are Weill Medical College of Cornell University, New York NY; H. Lee Moffitt Cancer Center, Tampa FL; Mayo Clinic, Rochester MN."). The conference appreciated that to answer certain questions, an unscreened comparison group was needed to supplement the three ongoing "one-armed" trials. The conference concluded, "comparative populations could be constructed by matching cases (e.g., age, smoking history, tumor classification) from populations currently enrolled in existing large studies or databases (e.g., PLCO screening trial, SEER). Such new methodologic approaches in response to a perceived public health emergency (i.e., 85% lung cancer mortality) may constitute an important precedent for public health research. The results of this innovative approach may guide public policy in formulating lung cancer screening recommendations and save a significant number of lives. As such, these activities merit high priority for creative funding support."

The complete text of the Consensus Statement from the First International Conference on Screening for Lung Cancer is appended to this report.

Funding Received Based Upon Work Supported by this Award

The archive and preliminary data from "Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers" provided essential support to our successful NIH application "The Biomarker Development Laboratory at Moffitt" (NCI-CA 84973, M. Tockman, PI, 1st year/Total award \$413,720/\$1,903,827).

Additional Research Opportunities Received Based Upon Work Supported by this Award

At the First International Conference on Screening for Lung Cancer, the principal investigators from Cornell and Mayo joined with me to coordinate our studies of helical CT and airway cell markers to improve our ability to combine our data and strengthen the power of our studies. The results of the combined studies may then be compared to a variety of external studies (e.g., Japanese) and control groups (e.g., NCI-PLCO trial) (see Consensus Statement).

The procedures and methods of this trial have been solicited by a newly formed consortium of NCI-Lung Cancer SPORE (Specialized Program of Research Excellence) investigators. This Lung Cancer Biomarkers and Chemoprevention Consortium now has preliminary NIH-NCI funding to begin planning for a national trial of helical CT and airway cell markers screening for lung cancer.

Appendix 1

THE FIRST INTERNATIONAL CONFERENCE ON SCREENING FOR LUNG CANCER

October 1 - 3, 1999

Weill Medical College of Cornell University, New York, NY
Consensus Statement

Summary

Lung cancer kills more individuals than cancers of the breast, colon, cervix and prostate combined. Recent scientific advances create an extraordinary potential to develop a lung cancer screening program that would prevent untimely deaths of vast numbers of current and former smokers who remain at high risk despite smoking cessation. The most promising of these scientific advances are rapid (single breath-hold helical) CT and computerized molecular analysis of airway cell markers (ACM). Each identifies lung cancers much earlier in their development than previously possible with conventional techniques and are likely to be complementary to each other in enhancing early detection of lung cancer. There is compelling evidence that use of these approaches can lead to high cure rates of lung cancer, a disease which currently has a dismal outcome.

The technology to implement these screening approaches currently exists and could rapidly be extended to offer screening to high-risk populations of smokers and former smokers. This creates an urgent need for research to evaluate how these techniques can best be utilized and the magnitude of the benefit they can create in order to allow appropriate public policy decisions about screening for lung cancer.

In December 1998, the International Conference on Prevention and Early Diagnosis of Lung Cancer, sponsored by the American Cancer Society (ACS), the Union International Contre Le Cancer (UICC), the Alliance for Lung Cancer Advocacy, Support, and Education (ALCASE) and other international organizations re-examined the recommendations against screening for lung cancer. The experts concluded that the information leading to these recommendations based on previous screening trials conducted more than two decades ago had a number of limitations and thus comprised an imperfect basis for current health policy. In light of emerging information and the enormous importance of the lung cancer problem, that conference recommended urgent reconsideration of issues surrounding early detection of lung cancer.

In response to this challenge, the first International Conference on Lung Cancer Screening was held in October 1999. A group of international experts in imaging, molecular diagnostics, pulmonology, oncology, epidemiology, clinical trial design, statistics, health care policy and patient advocacy met to address the issues central to lung cancer screening. The meeting was co-sponsored by the ACS, the National Cancer Institute, ALCASE, Weill Medical College of Cornell University and other organizations. The conference reviewed currently available data on lung cancer screening and engaged in intensive analyses of the implications with a view to attaining consensus with respect to the main issues surrounding early detection of lung cancer.

It was agreed that subsequent to the institutional policy statements not recommending screening for lung cancer, two important developments have occurred. Compelling evidence has continued to emerge over the past decades that resection of early lung cancer has major bearing on survival, and new techniques now provide for distinctly earlier detection of the disease. From this it follows that modern screening for lung cancer would save lives. Beyond this qualitative conclusion, there is an urgent need to learn about the magnitude of this effect.

It was recognized that more than 20,000 people have already participated in studies evaluating the efficacy of CT screening for lung cancer in the United States, Europe, Middle East, and Japan and more than 9000 using ACM. It was agreed that to further quantify the magnitude of the effect, the recommendation to use randomized controlled trials requires serious reconsideration for several reasons. The principal reasons among these are the high cost, long duration of such studies and the rapid advances in technology, together with existing visions of a less expensive and more rapid approach, one already being implemented by ongoing studies.

The conference agreed to form working groups and on a need to reconvene within six months to more closely review the ongoing studies and other interim developments.

Expanded Statement

Screening test(s) for lung cancer should be simple, inexpensive, noninvasive, and potentially widely available with a demonstrated acceptable level of sensitivity, specificity, and predictive value. Computed tomography and automated airway cell marker analysis were considered the most promising as well as being complementary (e.g., with regard to detection of central versus peripheral tumors and squamous versus adenocarcinoma histology). Other modalities were also considered but were deemed to either not meet the requirements stated above as they were still too early in their investigative course or more appropriate for diagnostic evaluation. These included: chest radiography (plain; digital, with or without computer-assisted diagnosis; energy-subtraction imaging, with or without such diagnostics), positron emission tomography, electrical impedance tomography imaging, magnetic resonance imaging, fluorescent light bronchoscopy, and CT virtual bronchoscopy.

Screening is only of value when it is linked with appropriate diagnostic interventions and treatment. The conference attendees concluded further evaluations of lung cancer screening should be conducted within the framework of an overall research program. Such a program would include standardization of diagnostic evaluation and treatment to minimize unnecessary diagnostics, invasive procedures and surgery. Further evaluation of treatment interventions should also be considered. It was decided that all future screening evaluation should be performed in a programmatic setting which includes outcome evaluation, quality assurance, standardized interpretation, diagnostic evaluation, organized reporting and results communications and education for physicians and screenees.

Very promising data from CT screening trials of about 20,000 screening subjects worldwide were presented, underscoring the need to evaluate these tools rapidly. To permit rapid evaluation, an infrastructure must be developed to allow for assessment of future screening modalities. Essential first steps include standardizing protocols, pooling of cohort data, and identifying

support mechanisms to ensure long-term clinical follow-up of vanguard populations. The sites at which CT screening is currently being performed in the United States are Weill Medical College of Cornell University, New York NY; H. Lee Moffitt Cancer Center, Tampa FL; Mayo Clinic, Rochester MN. Other centers abroad are Muenster University, Muenster, Germany; Hadassah Medical Center, Jerusalem, Israel; the National Cancer Center Hospital, Tokyo, Japan; and Shinshu University, Japan. It was also suggested that additional sites be added in an organized fashion to allow the rapid collection of sufficient screening data to refine and recommend definitive clinical trial design considerations. To supplement this information, comparative populations could be constructed by matching cases (e.g., age, smoking history, tumor classification) from populations currently enrolled in existing large studies or databases (e.g., PLCO screening trial, SEER). Such new methodologic approaches in response to a perceived public health emergency may constitute an important precedent for public health research. The results of this innovative approach may guide public policy in formulating lung cancer screening recommendations and save a significant number of lives. As such, these activities merit high priority for creative funding support.

Further research is necessary to determine optimum details for the target population characteristics (age, smoking history, etc.), periodicity of screening, noninvasive diagnostic algorithms after abnormal screening results, and invasive tissue sampling and treatment algorithms. In the conduct of these studies, consideration should be made to bank (store) images as well as certain samples from screened subjects for further study (e.g., sputum, blood, and exfoliated oral cells).

Three possible evaluation strategies were discussed in detail including the strengths, limitations, and implications of a choice of one design compared with the others. The currently recommended entry criteria for enrollment are: current or former (less than ten years since quitting) smokers, age 50 or more, and healthy enough to withstand thoracotomy (as determined by pulmonary function test). Such entry criteria are critical since an extension to other population groups may require a new trial according to the orthodox view.

The randomized clinical trial (RCT) with lung cancer mortality as its endpoint is the design that offers protection against the unknown influence of suspected biases such as selection bias, lead-time bias, length bias sampling, and over-diagnosis. It was recognized that such a trial could require 80,000 individuals to be randomized to an active population (AP) or a control group passive population (PP). A baseline screening and four annual repeat screenings with a follow-up period of 8 years after the last screen would be performed. Earliest opportunity for definitive data from such a trial, if it were to begin in 2000, is between 2009 and 2013 (a total of at least 3 rounds with a minimum of 5 years of follow-up). The large sample size is based on an unanticipated, but possibly worst case scenario of low benefit (10% mortality reduction) and high contamination in the PP.

The benefit of such a design is that the intervention would be evaluated without the influence of unknown biases. However, its high financial cost could lead to greater compromises (e.g., unknown threats to power due to erosion of the integrity of the randomization over time, in particular contamination; lack of acceptance of the study conclusion; new technology overtaking

the technology under evaluation, a potential lack of participants because of publicity about the presumed superior efficacy of CT).

An RCT using a surrogate endpoint still provides protection against selection bias but provides an earlier answer at lower costs. The surrogate must be a direct goal of screening (e.g., a more favorable stage-shift (TNM), tumor size, % positive nodes, histology) and strongly predictive of mortality. In such a trial, 40,000 subjects would be required. A baseline and four annual repeat screenings would be required with a follow-up period of at least 3 years after the last screen. The earliest opportunity for definitive data, if the trial were to begin in 2000, would be between 2005 and 2008. While issues related to access to control-group endpoints would need to be resolved, the benefits of the design include lower cost and more rapidly available data regarding the question of test efficacy. The concern about this approach includes the significant investment in time, the magnitude of potential error which may be difficult to quantify, lack of consensus about interpretation of end results-some policy makers may not accept the end result based on an approach that depends on predictors of mortality.

The third design, the non-comparative or quasi-comparative design, would require 8,000 to 10,000 individuals. It would require a baseline and a single annual repeat. Follow-up for both surrogate and mortality endpoints would be done, but only on the estimated 300 to 400 malignancies. The earliest opportunity for definitive data, if the study were to begin in 2000, would be 2002. Issues to be addressed would include access to comparison groups (PLCO screening study or older studies) and matching by relevant factors (e.g., histology, age, smoking history). The benefit of this design is that the data would be available more quickly and be obtained at a lower cost.

In summary, it was concluded that:

- An RCT with a death endpoint offers the most unbiased answer, but is not embraced with enthusiasm due to costs (mostly time) and the realistic appraisal that precision erodes over time. In some countries, a decision to offer lung cancer screening may require results from an RCT with a mortality endpoint.
- An RCT with surrogate endpoints is more attractive because of its inherent economy. Workgroup members were troubled by uncertainty that the intermediate endpoints accurately indicated mortality. It was also recognized that if one is willing to accept a study with surrogate endpoints, one should be able to accept a non-comparative study.
- At a minimum, the concept that funding for multiple designs should be considered rather than relying only on a single strategy to evaluate lung cancer screening. Further, the urgency of the public health problem warrants an immediate response by health agencies and professional organizations to support organized data collection and evaluation of the potential benefit and costs of all study designs to answer important questions.
- A non-comparative design could be used, following similar selection criteria. Data should be accumulated from the international sites currently performing helical CT studies and molecular analysis of airway cell markers. Common data collection procedures for all centers

should be organized. This effort should be multi-disciplinary in order to measure all end-results (detection data and follow-up) and address harms as well as benefits, including psychosocial issues. Models for efficacy and cost-effectiveness of surrogate endpoint and non-comparative designs should be developed. Efforts should be made to seek to answer questions about selection effect, lead time bias, length bias sampling (and overdiagnosis). Realistic estimates of the influence of potential biasing factors may result in greater applicability of alternative designs using comparative data (e.g., National Cancer Institute trial data such as PLCO, SEER registry, etc.)

It was felt that it was important to seek leadership from the appropriate specialty organizations (e.g., American College of Radiology, American College of Pathology, American Thoracic Society) to insure quality assurance for helical CT and computerized molecular analysis of airway cell markers, guidelines for these tests, subsequent interventions, and pathology

Evaluation of screening for lung cancer is critically dependent on accurate pathologic diagnosis of the disease. The majority of CT-detected malignancies are peripheral adenocarcinomas and, thus, patient specimens will include putative precursor lesions (i.e., pre-invasive lesions, solitary non-invasive non-mucinous bronchioloalveolar carcinomas and small invasive adenocarcinomas as well as occasional central airway squamous lesions). While not all cytologists and surgical pathologists are familiar with the current 1999 WHO/IASLC classification of lung tumors, the diagnosis of lung cancer is made with a high degree of accuracy. Difficulties in diagnosis are most often related to tumor sampling, the size of the sample and artifacts. The concept of overdiagnosis should not be confused with a false positive diagnosis of lung cancer by pathologists as these are, fortunately, exceedingly rare.

The early detection of lung cancer by helical CT and computerized molecular analysis of airway cell markers provides an important opportunity for radiologic-pathologic and clinical correlation. Since little is known about the clinical course of atypical adenomatous hyperplasia, solitary non-invasive non-mucinous bronchioloalveolar carcinoma, and early phase invasive adenocarcinomas, a single protocol for specimen handling and a central tissue registry are essential. An international panel will be utilized to reach consensus on difficult lesions, and the tissue bank will insure further clinical, radiographic, light microscopic, immunohistochemical and molecular studies of putative precursor lesions and small carcinomas. Through the collection of these lesions we can further our understanding of the biologic behavior of lung cancer.

**Breast Cancer Early Detection Study:
Feasibility of Detecting and Characterizing
Molecular and Antigenic Markers in
Fluid From Breast Nipple Aspirate
Principal Investigator: Gail L. Shaw, M.D.**

INTRODUCTION

Breast cancer causes over 40,000 deaths per year in the United States. This disease now affects one out of every nine women. The most effective method for improving a woman's chances of survival is early detection. However, new markers need to be identified that allow for even earlier identification of carcinogenic changes in breast tissue. The Breast Cancer Early Detection study attempts to identify and validate biomarkers that are more sensitive, specific and reproducible than are currently available, in conjunction with conventional mammography.

The purpose of the study is to determine if eligible women are willing and able to participate in an early detection study combining technologic evaluation and biomarker analysis, to determine whether these women are willing and able to provide nipple aspirate fluid (NAF) as well as cytologic specimens and portions of biopsies performed for clinical indications, to characterize the NAF by range of volume obtained and the ability to detect molecular and antigenic markers. The scope of the study will then extend to developing new methods to perform multiple assays on the NAF, to correlate the biomarker expression in breast tissue with expression in the NAF and to correlate the biomarker expression with mammographic patterns.

BODY

Task 1: Methods were developed to screen potentially eligible women using the scheduling database (EPIC) at the Lifetime Cancer Screening Center (LCS) both prospectively and retrospectively. Between 15 to 20 mammograms daily are scheduled at LCS, and diagnostic mammograms are performed every Wednesday (2-5 per week). All women scheduled for diagnostic mammograms were considered potentially eligible, and all women found to have mammographic abnormalities were then eligible. In addition, the LCS database collected family history data that could then be used to estimate a likelihood of eligibility applying the Gail model. Potentially eligible women were then approached if they returned for diagnostic mammography at LCS. Although all nurse practitioners, nurses and physicians at LCS as well as at the Comprehensive Breast Program at the Cancer Center were apprised of this study at least quarterly, a dedicated data manager was available only one day a week for independent recruitment activities. Recruitment was most successful on the day a data manager was available; referrals have not been forthcoming from the other days.

Since March 1999, when approved by the Army HRRB, 6 women have consented to participation. None of them yielded ductal fluid, and they are therefore considered off study. Since the study originally opened in August 1995, 72 eligible women agreed to participate and 17 women yielded 36 samples.

Task 2: Study accrual visits were scheduled to coincide with other scheduled appointments at LCS on Wednesdays. Wednesday is the day that diagnostic mammograms are performed at LCS and is also the day of the High Risk Breast Clinic which also includes a population of women at high risk for breast cancer potentially eligible for this study. A dedicated study data manager was not available on other days, and women were rarely able to return for a separate visit for study purposes only. On Wednesday participation was reasonable, but some women were unable and/or unwilling to prolong their stay to participate in the study, which required an additional 30-45 minutes. The inability to accrue adequate numbers of subjects and the inability to obtain fluid on the majority of subjects accrued has led to the conclusion that this study is not feasible in this setting at the present time, and the study has been closed to accrual.

Task 3: Nursing staff at LCS as well as the study data manager were trained by the PI in standard methods of breast nipple aspiration, and this was reinforced as new staff came on board. The dedicated study data manager became quite proficient, although our yield (24%) is still below the 50% reported by Petrakis. Appendix 1 details the clinical method used.

Task 4: Assays for epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) were developed and performed on the specimens collected. Data from these assays are presented in Appendix 2.

- IGF-1 assay: The measurement of IGF-1 was done with a commercial ELISA kit purchased from Diagnostic Systems Laboratories (Cat. No. DSL-1-5600). This ELISA assay kit is an enzymatically amplified "one step" sandwich-type immunoassay. The assay includes a simple extraction step in which IGF-1 is separated from its binding protein. In the assay, standards, controls and extracted samples are incubated with anti-IGF-1 mouse monoclonal antibody labeled with the enzyme horseradish peroxidase in microtitration wells coated with another anti-IGF-1 mouse monoclonal antibody. After incubation and washing, the well is incubated with the substrate tetramethylbenzidine. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of IGF-1 present. The amount of IGF-1 in the samples is interpolated from the absorbance measured from the set of included standards. Protein concentrations were measured using the Bio-Rad Bradford assay. IGF-1 levels were normalized to the amount of protein in the sample.
- EGF assay: The measurement of EGF was done with a commercial ELISA kit purchased from R&D Systems (Cat. No. DEG00, Quantikine®). The principle of the kit is that first a monoclonal antibody specific for EGF was precoated onto the microtiter dish. Standards and samples are pipetted into the wells and EGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for EGF is added to the well. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of EGF bound in the initial step. The color development is stopped and the intensity of the color is measured. The amount of EGF in the samples is interpolated from the absorbance measured from the set of included

standards. Protein concentrations were measured using the Bio-Rad Bradford assay. EGF levels were normalized to the amount of protein in the sample.

KEY RESEARCH ACCOMPLISHMENTS

- 36 NAF samples from 17 women out of 72 consented were obtained
- Analysis for EGF demonstrated feasibility of analyzing NAF for EGF
- No IGF-1 was detected in the NAF samples

REPORTABLE OUTCOMES

- Abstract presented at the First Santa Barbara Nipple Aspirate Fluid Conference, Santa Barbara, California, February 1999

APPENDIX 1: Nursing Methods

BREAST CANCER EARLY DETECTION STUDY

Medical History

For subjects scheduled to come for evaluation, a medical history questionnaire will be mailed to the individuals to complete and bring to their first visit.

Clinical Breast Exam (CBE) and Instruction in Breast Self Exam (BSE)

During initial evaluation, a complete history is taken and a clinical breast examination is performed. Instructions in performing a Breast Self Exam (BSE) are also given by the Nurse Practitioner. A mammogram will be ordered on women over 40 if not performed within the previous year. Complete data will also be collected regarding risk factors for breast cancer including parity, hormonal use, environmental and occupational exposures, medical history of breast diseases, family history, brief dietary history and medication usage.

Breast Duct Aspiration

Patients will be asked to have breast duct aspiration performed bilaterally at on-study and at follow-up visits.

Technique of Nipple Aspiration

A cup attached by plastic tubing to a 10 cc syringe will be placed over the cleansed nipple. While the subject compresses her breast between both hands the plunger of the syringe will be withdrawn to 10 cc and held until fluid appears at the nipple surface. This degree of suction approximates 300 mm Hg and is moderately greater than the suction developed by a nursing infant. The degree of suction is readily tolerated and only a few women complain of a pinching sensation. If no fluid appears within 15 seconds, the woman will be labeled a nonyielder for that attempt. Petrakis et al. have obtained NAF on at least one occasion from their cohort of 2,701 women and on multiple occasions from smaller cohorts with no complications of bruising or unacceptable discomfort. The fluid will be collected into capillary tubes. Women would be asked to consent to nipple aspiration bilaterally during the on-study and each subsequent visit. Results of the routine laboratory and radiology studies will be made available to referring physicians and participants. No results from the experimental assays of the NAF, cytologic or biopsy specimens will be made available to the participants since there are no reference levels for these markers.

Cytologic Needle Aspiration

Fine needle aspiration will be performed using sterile technique bilaterally in consenting women at on-study. Women may participate in the study even if they decline the needle aspiration.

How to label patient samples for the breast fluid study.

1. First write the protocol number, 11102, followed by a hyphen.
2. Next assign a number for the sample. The numbers are sequential starting with 1. This number is unique for each patient/visit. For example, if Mary Doe is the first patient, her number would be 11102-1 for her first visit. On a second visit, Mary Doe would get a new number.
3. If the sample is a nipple aspirate fluid, record this as an 'A'. For example, for Mary Doe's first visit, that sample would be 11102-1A. If Mary Doe also gave a fine needle aspirate sample, this would be noted as 11102-1B, where B denotes a fine needle aspirate sample.
4. If multiple samples are collected, say for example, from single ducts from both breasts or from different ducts from a single breast, the sample would be numbered sequentially after the letter denoting the type of sample. Using Mary Doe as an example, if she secreted nipple aspirate fluid from both breasts on her first visit, then the samples would be numbered as 11102-1A1 and 11102-1A2. The sequential numbers after the letter 'A' denotes the different samples. The inventory should define which breast produced which sample as well as which quadrant (clock face) of the nipple produced the sample. The same numbering system applies for multiple fine needle aspirate samples (but these would use a letter B to denote the type of sample).
5. When Mary Doe returns for another visit, any samples collected will be assigned a new number, whatever number is next after the last patient. If the next number would be 100, then for Mary Doe if a single nipple aspirate fluid sample is produced it would be labeled as 11102-A100.
6. If a patient does not produce a sample, they are still given a number, but the inventory denotes that no sample was obtained at this visit. For example, for Mary Doe's second visit (described in step 5), if no fluid was collected, her visit would still be noted as 11102-100. Notice no letter is assigned to this visit to denote no sample was collected.
7. For each visit, the patient should be queried about any changes in lifestyle or general health.
8. For each visit, the patient should be queried about oral contraceptive use.
9. For each visit, the patient should be queried about the use of cigarettes.
10. For each visit, the patient should be queried if she is using hormone replacement therapy.
11. For each visit, the patient should be queried about her current medication use.
12. For the first visit, a date of birth should be obtained.

Appendix 2: Figure Legends

Figure 1. Total protein concentration in NAF as determined by Bradford microassay (Bio-Rad) for each NAF sample.

Figure 2. EGF levels (pg/ml) as determined by sandwich ELISA for each NAF sample (R&D Systems, Quantikine kit, Catalog number DEG00).

Figure 3. EGF levels per milligram total protein for each NAF sample.

Figure 4. Total protein concentration in NAF from a single individual (AK) collected at three times over 12 months. Baseline samples, AK-1LB1 and AK-1RB1; 6 month samples, AK-1LB1, AK-2RB1, AK-2RLB2; 12 month samples, AK-3RB4 AND AK-3RB1.

Figure 5. EGF levels in NAF from a single individual (AK) collected at three times over 12 months. Baseline samples, AK-1LB1 and AK-1RB1; 6 month samples, AK-1LB1, AK-2RB1, AK-2RLB2; 12 month samples, AK-3RB4 AND AK-3RB1.

Figure 6. EGF levels per milligram total protein in NAF from a single individual (AK) collected at three times over 12 months. Baseline samples, AK-1LB1 and AK-1RB1; 6 month samples, AK-1LB1, AK-2RB1, AK-2RLB2; 12 month samples, AK-3RB4 AND AK-3RB1.

Figure 7. Total protein concentration in NAF from a single individual (CR) collected at two times over 6 months. Baseline, CR1A2a, CR-1A2 and CR-1A1; 6 month, CR2A2 and CR2A1.

Figure 8. EGF levels in NAF from a single individual (CR) collected at two times over 6 months. Baseline, CR1A2a, CR-1A2 and CR-1A1; 6 month, CR2A2 and CR2A1.

Figure 9. EGF levels per milligram total protein in NAF from a single individual (CR) collected at two times over 6 months. Baseline, CR1A2a, CR-1A2 and CR-1A1; 6 month, CR2A2 and CR2A1.

Figure 1: Protein Concentration

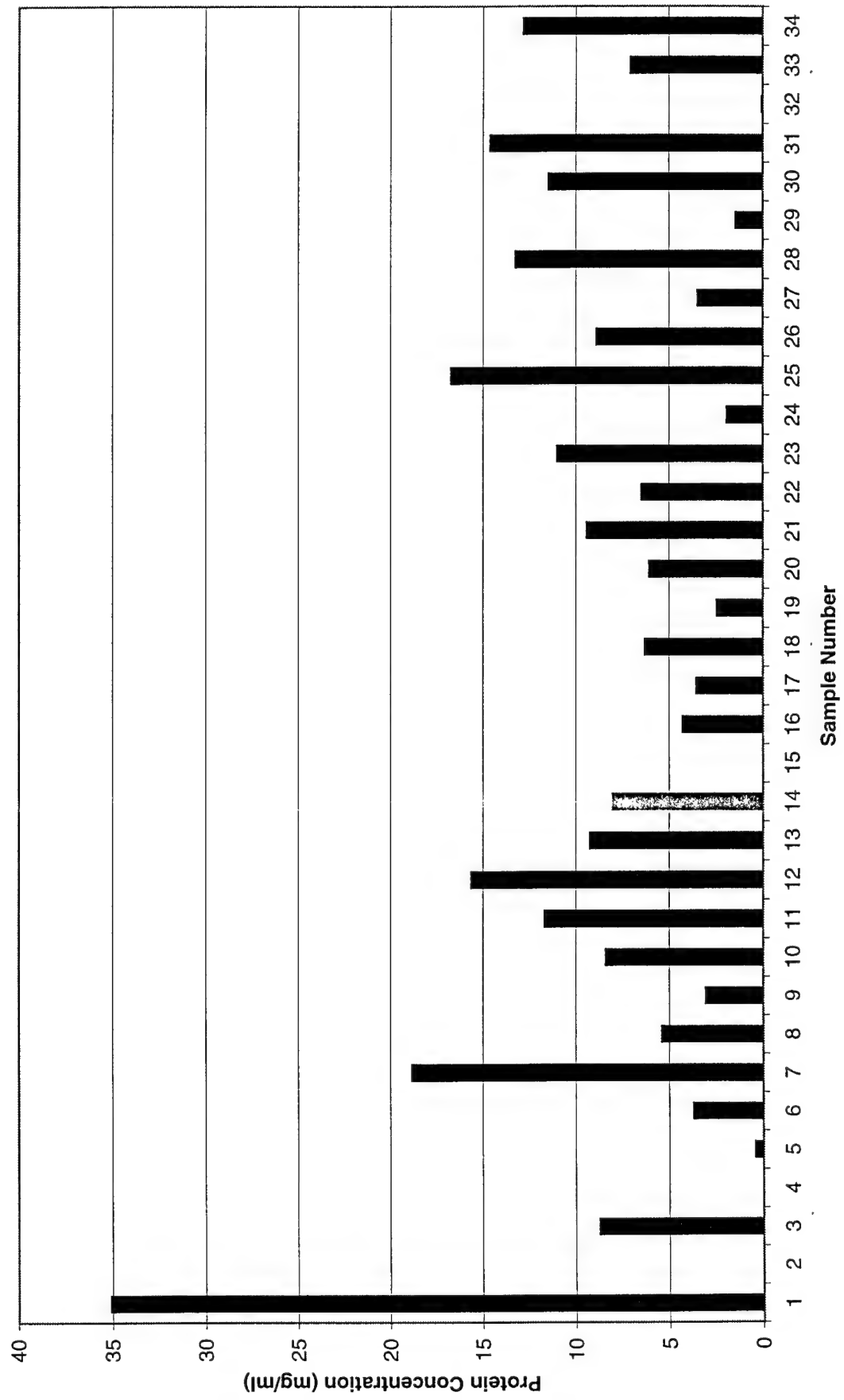


Figure 2: EGF Concentration

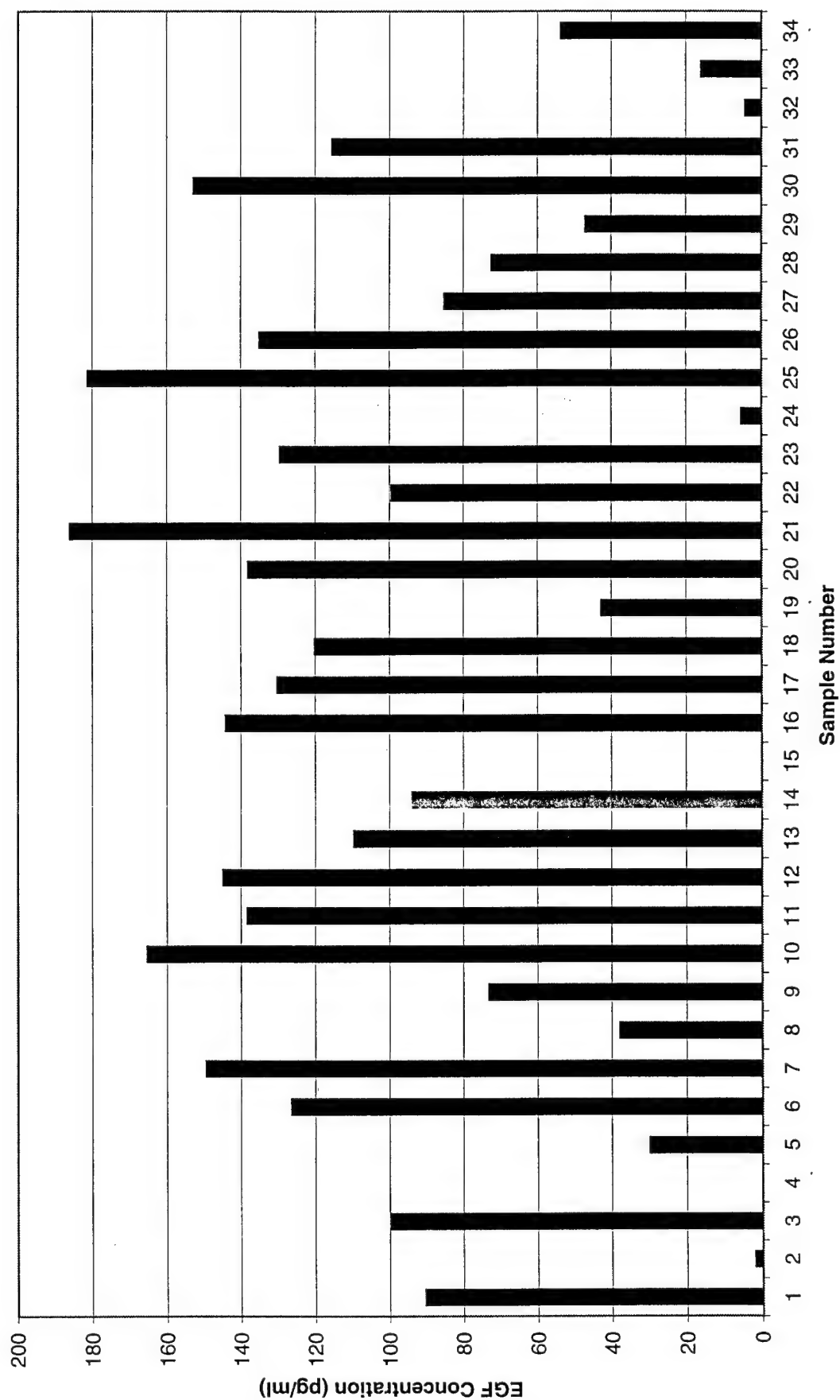


Figure 3: EGF

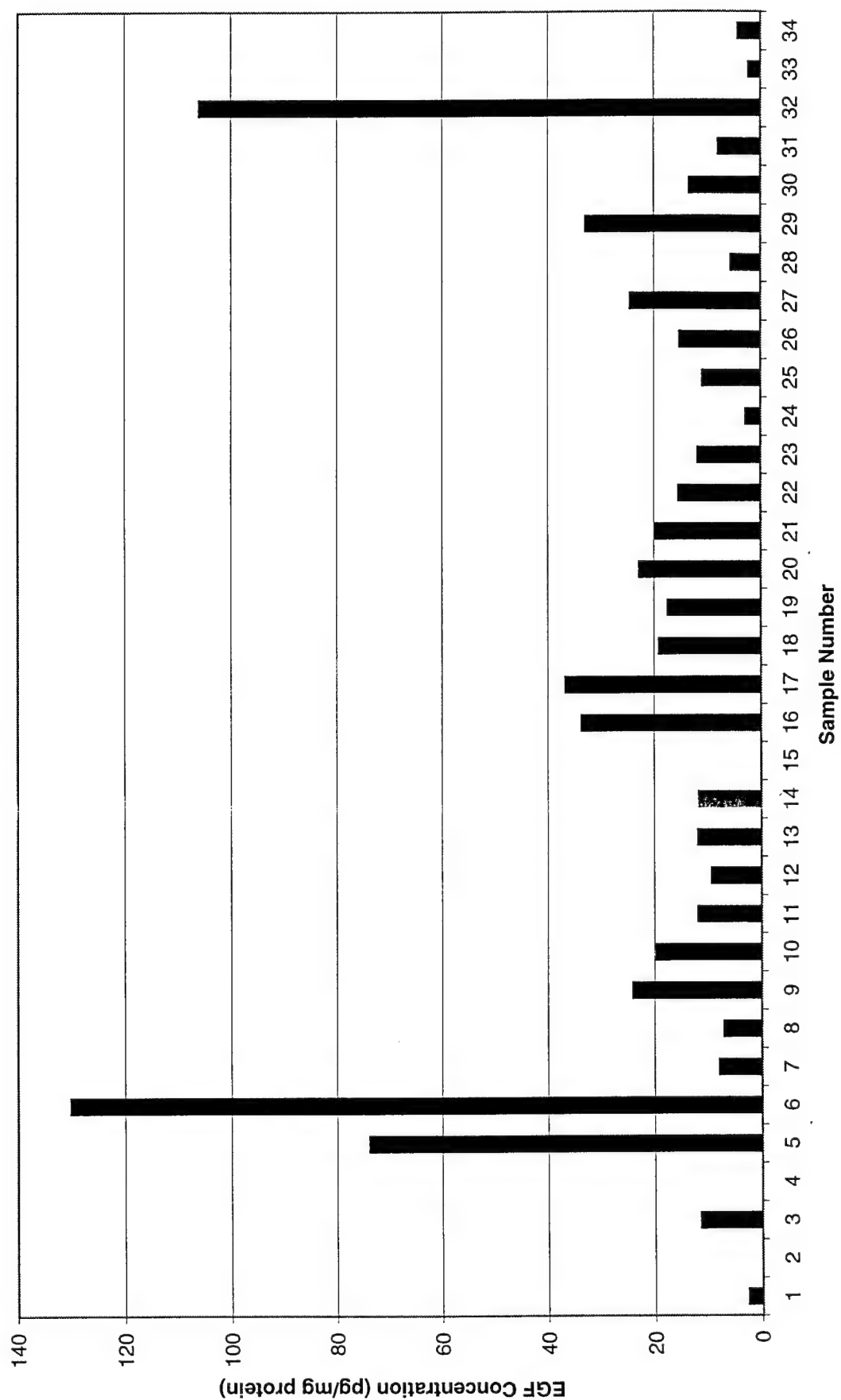
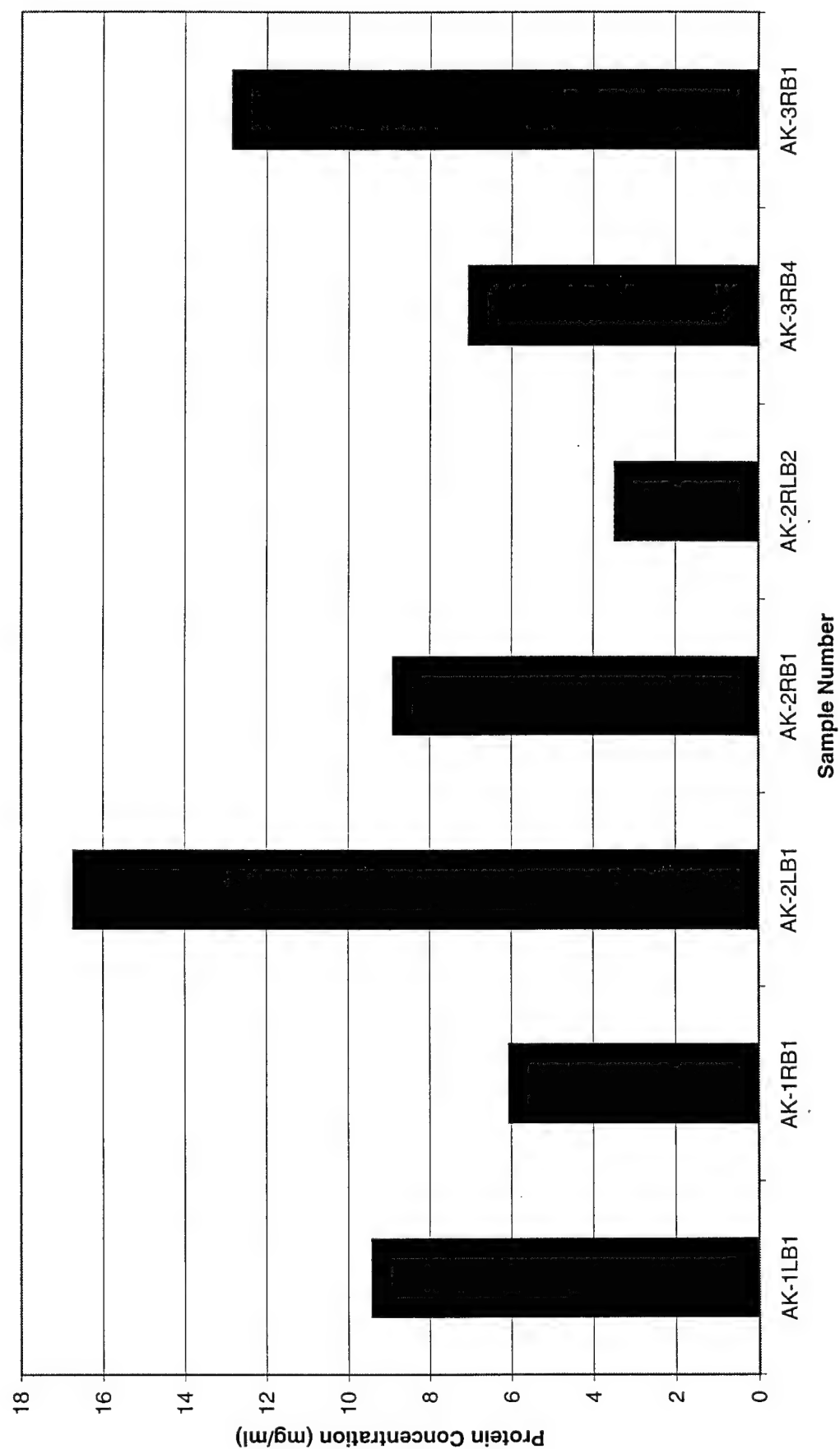
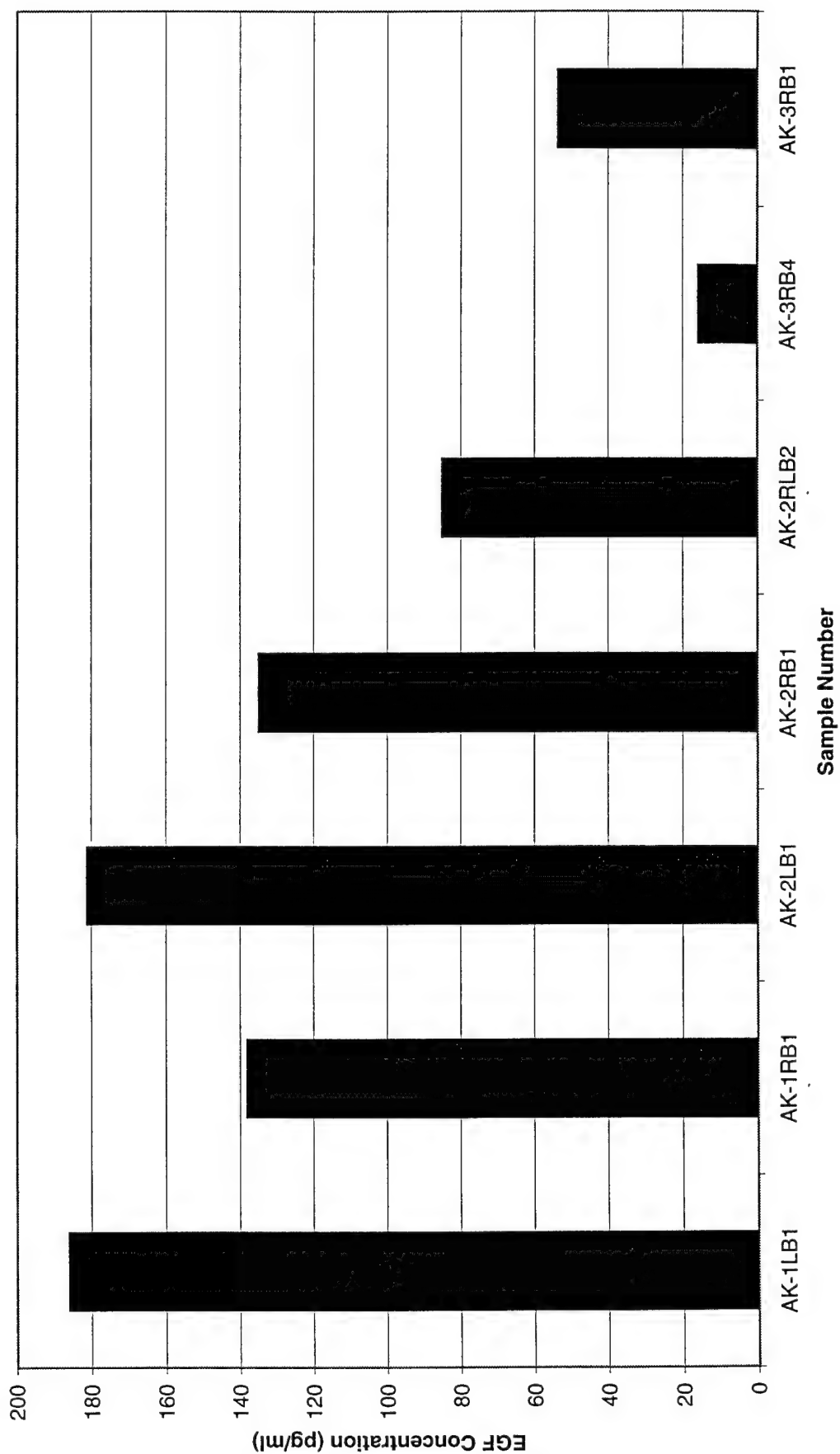


Figure 4: Total Protein
Baseline, 6 and 12 months



**Figure 5: EGF Levels
Baseline, 6 and 12 months**



**Figure 6: EGF and Total Protein
Baseline, 6 and 12 months**

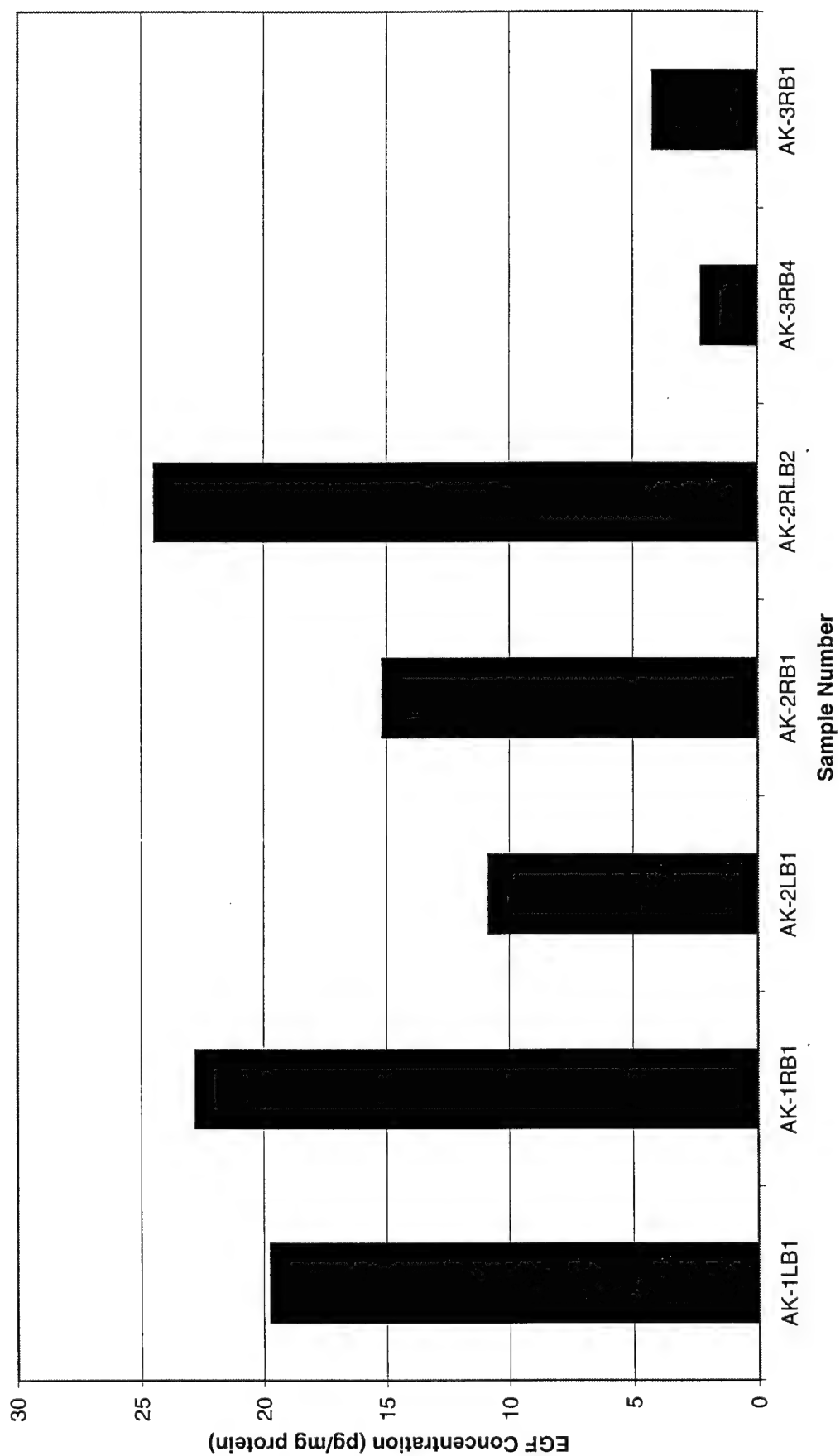


Figure 7: Total Protein-over 6 months

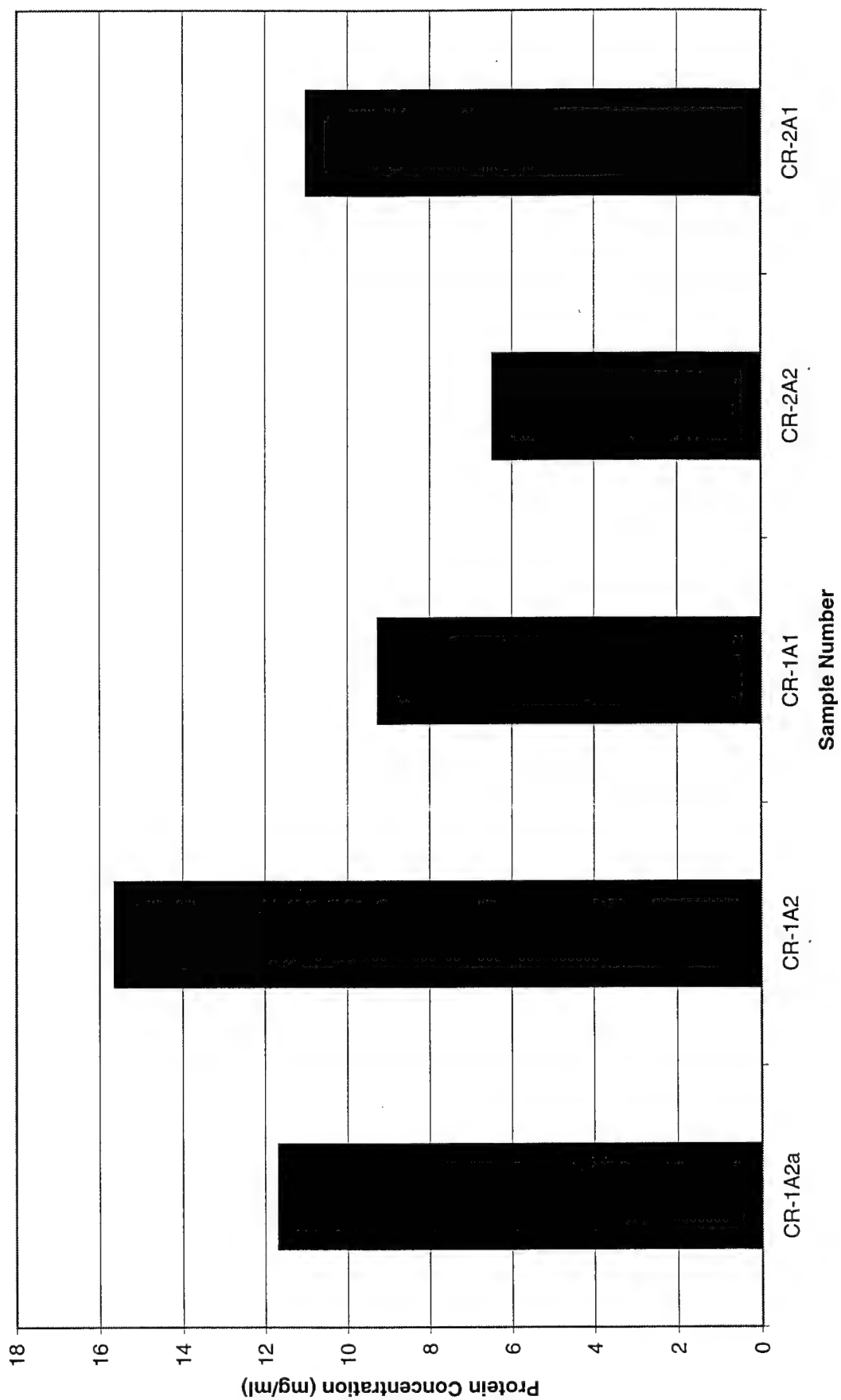


Figure 8: EGF Levels-over 6 months

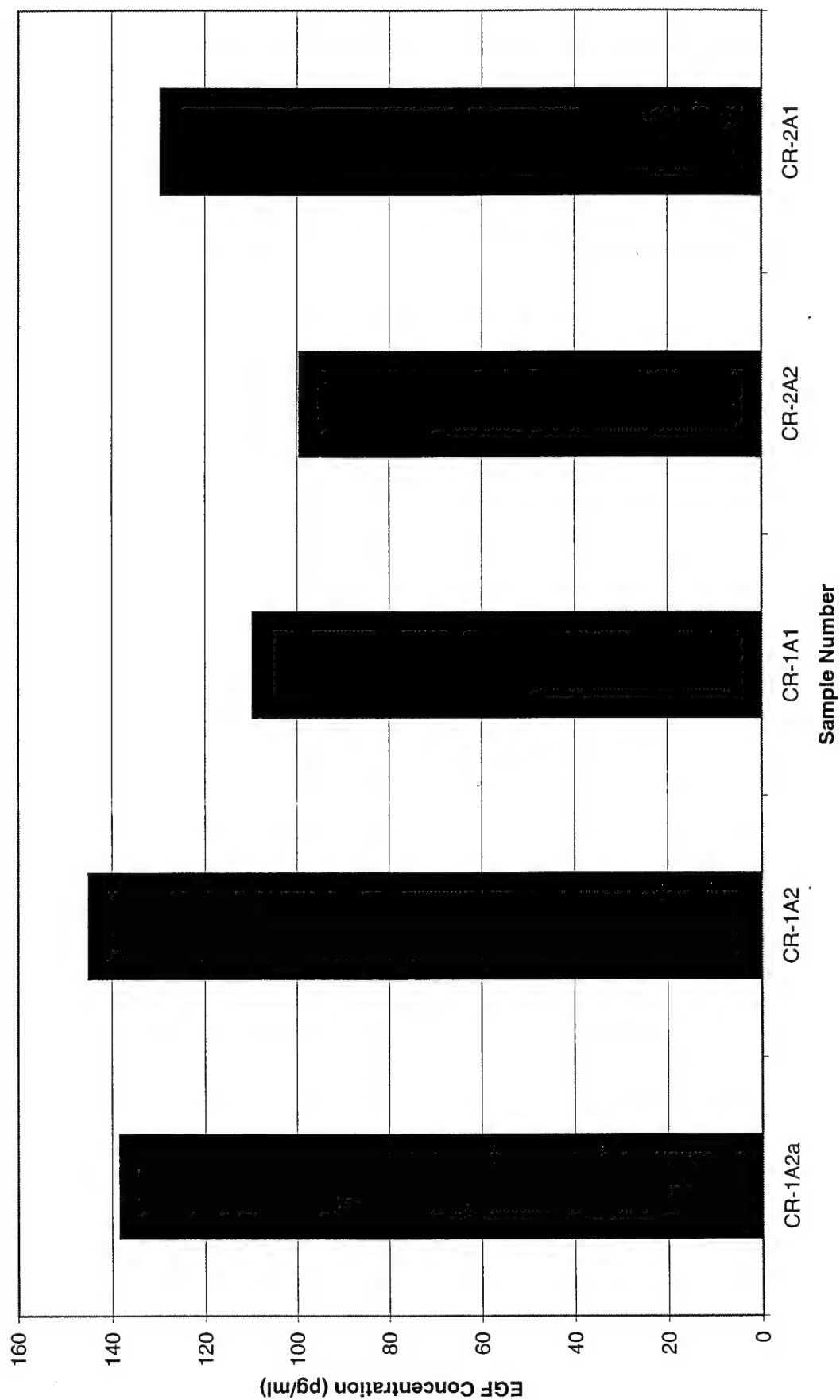
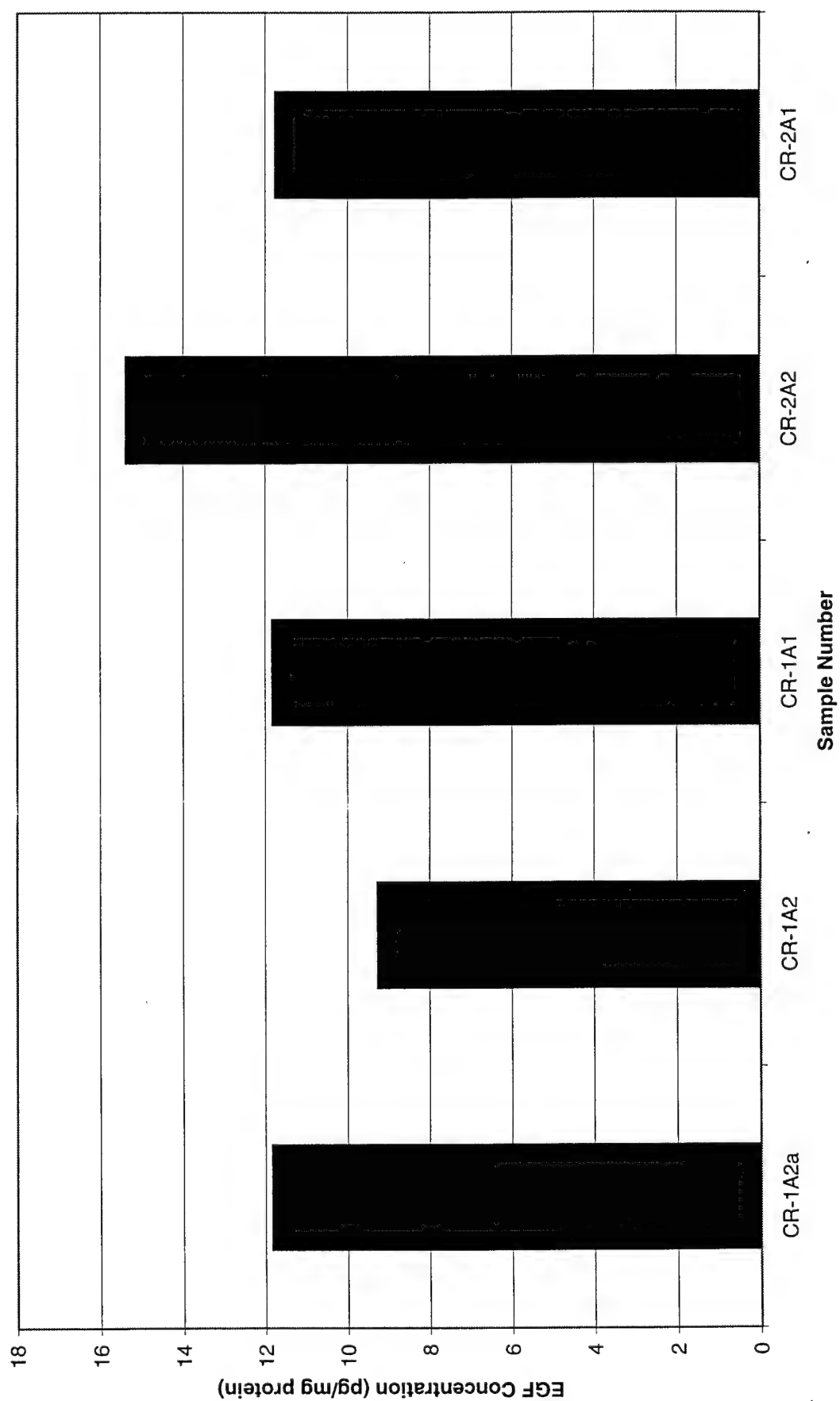


Figure 9: EGF and Total Protein-over 6 months



Linkage Analysis of Hereditary Prostate Cancer Families

Principal Investigator: Rebecca Sutphen, M.D.

I. Introduction

This study is a genetic linkage analysis of families in which more than one individual has been diagnosed with prostate cancer, in order to determine whether the prostate cancer in each family appears to be linked to one of the specific chromosome regions implicated recently in familial prostate cancer (1q24-25, 1q42.2-43, 1p36, Xq27-28) indicating the location of a prostate cancer susceptibility gene. Men with prostate cancer who have been treated at Moffitt and who have a family history of prostate cancer are invited to participate in this study through a letter signed by their Moffitt physician and the Principal Investigator. After providing informed consent for participation, subjects complete a detailed questionnaire which provides personal and family history. Based on review of the questionnaire, families are determined to be eligible or ineligible for blood sampling and genetic laboratory linkage analysis based primarily on the number of living affected family members who are willing to provide blood samples.

II. Body

Moffitt Cancer Registry data identified 133 men with prostate cancer who were treated at Moffitt and reported a family history of prostate cancer. One hundred thirteen men were living at the time of contact. Eighty-six were identified as having family history suggestive of hereditary prostate cancer, defined as 1) at least three affected members with prostate cancer and/or 2) at least 2 affected members with prostate cancer with at least one individual diagnosed at age 55 or younger.

The study received Institutional Review Board approval in June 1998. A Certificate of Confidentiality from the National Institute of Mental Health was obtained in September 1998. Of the 86 men identified through registry data, 39 who reported at least 3 affected family members were initially invited to participate. Seventeen subjects were enrolled in the study. Review of the questionnaires of enrolled men resulted in identification of 3 eligible families. Blood samples have been obtained from the 3 patients, and collection of blood samples from family members is in process.

On April 9, 1999, letters of invitation were mailed to the remaining 47 men from the original set of 86 patients. Ten of these men have completed questionnaires, of which 6 represent eligible families based on number of living affected relatives. Blood samples have been obtained on these 6 men, and collection of blood samples from family members is in process. An additional 5 men have agreed to participate and provided written informed consent, but have not yet returned completed questionnaires.

Letters of invitation were sent to the remaining 38 men of the 113 total living patients. Three men declined participation and 8 men reported that all affected relatives are deceased. It has not been possible to contact 8 subjects. Nineteen subjects were in the process of being contacted to determine interest in participation, answer questions and determine eligibility.

In summary, we have enrolled and obtained blood samples from 9 men of 133 identified (113 living) patients with prostate cancer and a family history of prostate cancer through the Moffitt Cancer Registry. Recommended changes to the study include 1) collection of tumor tissue as a source of DNA for the linkage analysis from deceased relatives of appropriate subjects and 2) expansion to the regional military population through the two local veterans' hospitals.

III. Key Research Accomplishments

- A. We have enrolled 9 men with a history suggestive of inherited prostate cancer in a genetic linkage analysis study.

IV. Reportable Outcomes

- A. We have developed study questionnaires which utilize an automated data entry system to facilitate data entry and data review.
- B. We have developed a database for the study which is capable of receiving data automatically from questionnaires which are scanned or faxed into the database.

The Specific Role of Genistein in Estrogen Metabolism

Principal Investigator: Nagi Kumar, Ph.D., R.D.

INTRODUCTION

This study is based on the research that has focused on isoflavones, specifically genistein, that have weak estrogenic and anti-estrogenic properties, similar to the chemopreventive agent Tamoxifen. Isoflavonoid phytoestrogens found in soy products have also been shown to increase serum SHBG which then decreases the bioavailability of estrogen and testosterone, since higher levels of SHBG result in lowering of free estradiol and free testosterone. This may also be due to the weak estrogenic effect of phytoestrogens which, like Tamoxifen, stimulate the synthesis of SHBG in the liver. Several studies also indicate that phytoestrogens reduce the bioavailability of estrogens by actually occupying estrogen binding sites exerting a weak estrogenic effect (anti-estrogenic) decreasing the availability of estrogen receptors to endogenous, biologically active estrogen. In another study examining the effects of a soy protein diet on the menstrual cycle, consumption of 60 gms of soy protein over a 1 month period led to a significant increase in follicular phase length and delay in menstruation, further demonstrating the ability of isoflavones to alter the hormonal milieu with potentially beneficial effects similar to those observed with Tamoxifen. In addition, research on in vitro and in vivo models of cancer found that in 74% of the studies using animal models, the proliferation of mammary tumors was significantly reduced with genistein. In vitro, genistein also had an inhibitory effect on human tumor cell lines. This inhibitory effect on in vitro cell cultures is attributed to its influence on tyrosine kinase activity. It appears that genistein acts to impair the signal transduction pathway from tyrosine kinase receptors, a step which is necessary for mitosis of human breast cancer cells. In another study of plant estrogens on estrogen sensitive cancer cells, genistein was found to compete with estradiol binding to estrogen receptors. It has also been postulated that plant lignans and isoflavonoid phytoestrogens may decrease aromatase activity, a cytochrome P450 enzyme, thus decreasing conversion of androgens to estrone and estradiol, which may then play a protective role in the development of hormone related cancers. Although genistein has many interesting anticarcinogenic properties, we intend to focus initially on its effects specific to hormone metabolism and antiproliferative properties specific to breast and prostate carcinogenesis.

Although there are some epidemiological and several animal studies supporting the cancer preventative qualities of soy products there have been few definitive, prospective clinical studies testing the *exclusive* effects of specific isoflavones on biomarkers that are implicated in the initiation and promotion of breast cancer. It is important to determine whether change in sex hormones, specifically serum sex-hormone binding globulin (SHBG), estrone and free estradiol levels vary with increased intake of genistein. If increased intake of the isoflavone genistein produces an elevation in serum SHBG and a decrease in serum levels of free estradiol and estrone, we will be able to clarify the action of isoflavones on serum sex-hormone metabolism and its role in the reduction of the biologically active form of estrogen. If increased intake of the isoflavone genistein alters the sex hormone concentration, bioavailability or metabolism, manipulation of the diet by adding rather than restricting food may reduce breast cancer risk. In addition, as a result of this study, dietary guidelines (e.g., National Research Council, 1989) may be refined, and the credibility of such recommendations will be enhanced. Based on the results of

this study, prophylactic therapies using dietary supplements such as genistein, with practically no side effects, may also be used to replace the more controversial therapeutic, hormonal supplementation regimens that are currently used for breast cancer risk reduction. In addition, studies examining the effect of genistein supplementation, combined with fat reduction and the effect of such a dietary regimen on both body composition, weight and sex-hormones can be examined with respect to hormonal cancer risk reduction. Although conducted on a female population, based on the current evidence in the literature, it is anticipated that this proposed study will generate results which parallel effects in prostate cancer risk reduction.

PURPOSE

The main purpose of the study was to evaluate the individual effectiveness of supplementing a group of pre-menopausal, breast cancer-free women with a dietary supplement of the isoflavone genistein (40mgs/day) in producing a change in sex-hormones that are implicated in the initiation and promotion of breast cancer, such as a decrease in serum-free estradiol and estrone and increase in serum SHBG levels.

ACCOMPLISHMENTS

As planned and described in the Statement of Work:

Task I: Start up: printing forms, screening, contact individuals regarding recruitment:

The first two to three months of the funding period were utilized to prepare all instruments, consent form packages and start the recruitment talk. This was accomplished successfully through recruiting subjects from the Tampa Bay area by public appeal. advertisements in the *Tampa Tribune* and in the news media. We have contacted and screened 303 women to date for this study.

Task II: Recruitment, data collection, process and construct data files:

- a. Of the 303 that we initially contacted, we have screened 260 women for eligibility, and recruited 63 subjects in the study.
- b. Eighty-four (84) pre-menopausal, breast cancer-free, omnivorous women, of all races and ethnicity, between ages 25 and 55 inclusive, at first screening contact, residing in the study area for the entire period of the study after randomization, and providing written informed consent were admitted in the study, of whom 52 have completed the study and 11 are currently active, pending completion in December 1999.
- c. An unanticipated drop out rate of 30% was observed, with over 21 subjects dropping out of the study. Over 17 subjects withdrew as they were unable to stay on the supplement for a prolonged time, one (1) complained of headaches, one (1) subject became pregnant and two (2) reported diarrhea with the products used in the study. The predominant criteria that excluded women were perimenopausal status, living outside the Tampa area or consumption of soy or other supplements at first screening.
- d. Upon eligibility, consent was obtained from all subjects.

Upon enrollment, the following baseline information was obtained from all subjects admitted to the study:

1. Confirmation of the accuracy of eligibility information, including the 4-day diet records and using an initial screening form.
2. Demographic information, personal and medical history, hormonal and reproductive history, exercise, smoking and alcohol use history obtained by an RD using the Epidemiological Questionnaire. The instrument will be administered at baseline.
3. Anthropometric measurements such as subject's height, weight, skinfold and circumference measurements. Anthropometric measurements were obtained during the visit to the clinic to see the RD. Weight and body fat distribution charts are maintained on all subjects for the study period.
4. Blood samples were drawn into heparinized tubes in a non-fasting state at the same time of day, between 7:00 AM and 12:00 PM, for each individual. Thirty mL of blood was taken from subjects during the mid-follicular phase of their menstrual cycle or 4-7 days after the start of menstrual flow. These samples were taken at baseline before intervention and again at the end of the study period. Subjects were instructed to call or page the Project Director on Day 1 of their menstrual cycle. An appointment was scheduled for them to meet with the RD and have their blood drawn.
5. After venipuncture is performed the blood will be centrifuged, the plasma separated, ascorbic acid and sodium azide (0.1% final concentration for both) added. The samples collected from each subject are stored at -20 degrees Centigrade until they are packed in dry ice and shipped to Corning Nichols Institute. Plasma levels of estrone, estradiol and sex-hormone-binding-globulin(SHBG) were determined.
6. The participants were provided with a 4-day diet record (FDFR) and instructed on reporting food intake, including weights/measures and methods of preparation of foods consumed using standard food models. Written instructions will accompany subjects with instructions on how to complete a FDFR. Subjects will be instructed to call the RD if questions arise as to how specific specialty foods need to be documented. The trialists paid special attention to subjects from ethnic backgrounds, specific to the Tampa Bay area. Specific food models, handouts, videos, specific to the ethnic groups that are currently used by the trialists were be used. In addition, ethnic differences in nutritional intake between participants will be dealt with by stratified randomization. The RD made telephone follow-up calls during one of the four days assigned for the first week of the intervention.
7. The trialists and the subjects in the study will be blinded as to the nature of the product. Having been assigned to groups A or B, subjects were instructed and provided information on:
 - a. Introduction to the product and mixing instructions which are the same for both the experimental and the control group.
 - b. Importance of compliance with and completion of 4-day food records.

- c. Compliance and completion of Nutritional Symptoms Scale.
 - d. Attendance to clinic by the 2 groups to obtain weekly supplements, self-monitoring tools and to submit to the research staff completed self-monitoring tools and leftover supplements, if any.
 - e. Compliance to diet without altering or reducing other foods.
8. Changes may be anticipated in stool frequency or GI discomfort. A pre-validated Nutritional Symptoms Scale was used to monitor GI symptoms during intake of supplements.
9. Menstrual histories were obtained from all subjects at baseline. The length of the menstrual cycle, both the follicular and luteal phase will be monitored by use of a commercial kit, First Response Ovulation Predictor (Carter-Wallace, Cranbury, NJ), which will provide qualitative information on when ovulation occurred. Subjects were instructed as to the use of the Ovulation predictor kit. Date of Day 1 of menses was required to be documented by the subjects on the Nutritional Symptoms Scale. The follicular phase will be defined as the first day of menstrual cycle to the day that the luteinizing hormone surge was noted. The luteal phase will be defined as from the day after the luteinizing hormone surge was noted to the day before the onset of the next menstrual cycle. This close monitoring of the menstrual cycles will enable us to observe the possible effects of isoflavone supplementation on cycle lengths.
10. A Participant Tracking Form was used to monitor all activities and variables observed during the study period. Activities of each participant is vital for the study such as use of supplements, compliance to all monitors. This form, will in addition, serve as a checklist to monitor these variables for the Project Director:
- a. Follow-up interviews for data collection periods at mid-point and post-completion of interventions have been completed for 52 subjects and are ongoing for the remaining 10.
 - b. Weekly visits to the cancer center to obtain supplements and submission of monitoring instruments.
 - c. Shipping of completed patient's blood sample for hormonal assays is ongoing

Technical Problems Encountered and How Our Approach Was Modified

Supplement: Although the genistein and milk powder supplement that we initially received from the manufacturer was taste tested in our pilot study, it was felt by the research group that the product tasted "chalky", had no flavor, with poor palatability, as it had been on the shelf for over 8 months. This presented a concern to the researchers as compliance over 12 weeks will present a challenge to the researchers and study subjects. This was reported to Protein Technologies, who then replaced the entire batch of genistein and milk powder with a vanilla flavor. Consequently, the start date for the study was prolonged by 8 weeks, taking us to the month of October 1997. Based on our experience and as reported in the literature, there was a consensus among the research team that the months of October, November and December would not be ideal dates to start a dietary intervention study, as subjects were required to consume two packages per day and not alter their usual intake. As these months fall during the holidays, apart

from compliance to the supplements, unusually high intake of other nutrients may have seriously affected the results of the study. Thus, our first subject was entered into the study on January 5, 1998.

Timing for completion of hormonal assays: To ensure sufficient funding for baseline and post-intervention hormonal assays, all baseline serum samples are currently labeled, packaged and stored appropriately at the Cancer Center laboratory. When subjects complete the study, their baseline and post-intervention samples will be picked up by the laboratory at the end of the study period for each subject. This will eliminate the cost of baseline assays on subjects who may then withdraw from the study. The response to the study from the Tampa Bay area was overwhelming. Thus, although the attrition rate is higher than anticipated (30%), we are confident that we will obtain the numbers needed for the study. All other procedures as established in the original proposal were effective and will be implemented for the last year. No results are available at this time.

REPORTABLE OUTCOMES

Data files have been created and data entry has been completed for the 52 subjects who have completed the study. Analysis of data on 52 subjects indicates that experimental group subjects, as hypothesized, experienced longer menstrual cycles, shorter follicular cycles, decreased free estradiol, and increased sex-hormone binding globulin from baseline to post intervention with genistein compared to the placebo group.

The preliminary results of this study were presented at the following International and National Scientific Meetings:

1. *Proc. of the 4th Annual Symposium on Predictive Oncology and Therapy Sponsored by the International Society for Preventive Oncology*; Nice, France, 1998
2. Cancer Control Branch, NCI, Bethesda, MD, 1999
3. *Proc. American Institute of Cancer Research Annual Meeting*, Washington DC, 1999

CONCLUSIONS

We plan to continue to complete recruiting to obtain a sample size of 68 subjects as proposed. The sample size in each group will be chosen so that the above comparisons for each time will have a power of 0.80 if the true difference between mean hormone levels of the two groups is 0.7 standard deviations. These will be two-sided pooled t tests with alpha equal 0.05. This will require 34 subjects in each group for a total of 68. Although we planned allowing for 10% dropouts/inevaluable, we will now modify this to allow for 30% drop out rate and recruit 90 subjects to the study. Based on standard deviation reported by Rose et. al, we will have 80% power for a difference of 147 pmol/L in mean estradiol levels, 108 pmol/L in mean estrone levels and 25 nmol/L in SHBG levels. The sample size justification was driven by the sample size we could reasonable expect. We show that this sample size enables us to have acceptable power for differences that may indeed exist and that would be medically significant. It is not well known what the smallest medically significant differences are.

We plan to complete data entry and analyze the data with all data prior to reporting conclusions in this study and continue to test the following hypotheses:

1. Supplementation with the isoflavone genistein will produce an increase in serum levels of sex-hormone binding globulin (SHBG). This increase will exceed that of a placebo control group. A pooled t-test will compare the two groups' mean SHBG levels at the conclusion of Phase II.
2. Supplementation with the isoflavone genistein will produce a decrease in serum-free estradiol and estrone sulphate. These decreases will exceed that of a placebo control group. Pooled t-tests will compare the two groups' mean estradiol and estrone levels at the conclusion of Phase II.

OTHER RESEARCH QUESTIONS

1. Does supplementation of diet with genistein affect length of menstrual cycle?
At the conclusion of Phase II, the length of each woman's last menstrual cycle will be recorded. The two groups' mean length of menstrual cycle lengths will be compared with a pooled t-test.
2. Will women whose diets are supplemented with genistein alter their nutritional selection of specific other nutrients such as fats, fiber, vitamin A and C?
Each woman's final 4-day diet record will be analyzed for fat, fiber, vitamins A and C intake. Pooled t-tests will be used to compare the two groups for intake of each nutrient.
3. Will supplementation with genistein affect anthropometric parameters such as QI, body fat and body fat distribution measurements?
Mean body fat ratio, QI and body weight changes of the two groups at the conclusion of Phase II will be compared by pooled t tests.
4. Will supplementation with genistein produce nutritional symptoms?
The proportion of women reporting increased stool frequency and GI distress in the two groups will be compared by Chi-square tests. If, for either symptom, the number experiencing it is less than five, Fisher's Exact Test will be used instead.

Pooled t-tests are justified in this case. Even if the data are only approximately normally distributed, the test is well known to be quite robust with respect to the normality assumption. These tests will be two-sided.

Multivariate repeated measures ANOVA will be performed on each variable with time as the repetition variable and treatment group as class variable. "Time effect", "group effect" and "time by group" interaction will be tested. If this last effect is significant, then "time effect" will only be reported separately for each group and "group effect" will only be reported separately for each time.

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer

Principal Investigator: Nagi Kumar, Ph.D., R.D.

INTRODUCTION

Several natural anticarcinogens have now been identified in soybeans, such as: protease inhibitors, phytate, phytosterols, saponins, lignans and isoflavones. However, our focus will be on the components solely unique to soybeans, the isoflavones, also referred to as phytoestrogens, due to their similarity to estrogen both structurally and functionally. Among the isoflavones, diadzein and genistein are the major forms present in soybeans. After structural modifications by intestinal bacteria, isoflavones are converted to compounds which possess weak estrogenic and anti-estrogenic properties. The chemopreventive agent Tamoxifen, which has both estrogenic and anti-estrogenic properties, is structurally related and may act in much the same way as isoflavones. These substances have been shown to not only influence hormonal metabolism but also intracellular enzymes, protein synthesis, growth factor action, cell proliferation and angiogenesis. Genistein has received a great deal of attention due to its interesting antiproliferative, estrogenic and anti-estrogenic effects. Genistein also showed the highest concentration of all phytoestrogens present in the urine of Japanese men and women consuming their typical diet which is rich in soy products. In a recent review of research regarding the effect of genistein on in vitro and in vivo models of cancer it was found that, in 74% of the studies using animal models, the proliferation of mammary and prostatic tumors was significantly reduced with genistein. In vitro, genistein also had an inhibitory effect on human tumor cell lines. In another study of plant estrogens on estrogen sensitive cancer cells, genistein was found to compete with estradiol binding to estrogen receptors. It has also been postulated that plant lignans and isoflavonoid phytoestrogens may decrease aromatase activity, a cytochrome P450 enzyme thus decreasing conversion of androgens to estrone and estradiol, which may then play a protective role in the development of hormone related cancers. Although genistein has many interesting anticarcinogenic properties, we intend to focus on its effects specific to hormone metabolism and biological activity specific to prostate carcinogenesis. Phytoestrogens found in soy products have been shown to increase serum SHBG via increased hepatic synthesis which, as a result, then decreases the bioavailability of testosterone. In addition, although phytoestrogens have been shown to have an antiestrogenic effect in a high estrogenic environment, it has been postulated by at least one researcher that they exert a proestrogenic effect in a low estrogenic environment. We postulate that genistein can theoretically increase production of SHBG by the liver, bind to biologically active testosterone, thus lower free testosterone levels and its bioavailability to target prostatic cells, and with its proestrogenic property in the low estrogenic environment increase the levels of estradiol, thus producing the estrogen/androgen synergy that is essential for protection from prostate cancer, and wish to initially observe serum biomarkers of free testosterone, estradiol, and sex-hormone binding globulin levels in addition to markers of prostatic proliferation such as percentage free and total Prostate Specific Antigens.

SIGNIFICANCE

Although there are some epidemiological and several animal studies supporting the cancer preventative qualities of soy products there have been no definitive, prospective clinical studies testing the *exclusive* effects of specific isoflavones on biomarkers that are implicated in the promotion of prostate cancer. It is important to determine whether change in sex hormones, specifically serum sex-hormone binding globulin (SHBG), free testosterone and estradiol levels vary with increased intake of genistein. If genistein can increase production of SHBG by the liver, bind to biologically active testosterone, thus lower free testosterone levels and its bioavailability to target prostatic cells, and with its proestrogenic property in the low estrogenic environment increase the levels of estradiol, thus producing the estrogen/androgen synergy that is essential for protection from prostate cancer, we will be able to clarify the action of isoflavones on serum sex-hormone metabolism and its role in the reduction of the biologically active form of testosterone. If increased intake of the isoflavone genistein alters the sex hormone milieu and the bioavailability to target tissues, this should theoretically reduce or halt proliferation, as observed by changes in prostate-specific antigen. Thus manipulation of the diet by the addition of genistein may reduce further progression of prostate cancer in this population. Based on the results of this study, prophylactic therapies using dietary supplements such as genistein, with practically no side effects, may also be used for high risk populations and replace the more controversial therapeutic hormonal supplementation regimens that are currently used for prostate cancer risk reduction.

PURPOSE

The purpose of this study is to evaluate the individual effectiveness of supplementing a group of grade 1-2 prostate cancer patients with a dietary supplement of the isoflavone, genistein (60mgs/day) in producing a change in risk parameters that are implicated in the promotion of prostate cancer, such as decrease in free testosterone, and increase in sex-hormone binding globulin and estradiol, and decrease in proliferation as indicated by decreasing total and percentage free Prostate Specific Antigens.

ACCOMPLISHMENTS

As planned and outlined in our Statement of Work, we have accomplished the following :

Task 1: Recruitment and Data Collection

- a. We have pre-screened 28 and recruited 20 patients diagnosed with grade 1-2 prostate cancer who were consecutively admitted to the Prostate Program.
- b. Upon eligibility, consent was obtained from all subjects. Upon enrollment, the following baseline information has been obtained from all subjects admitted to the study.
- c. Confirmation of the accuracy of eligibility information, including the 4-day diet records and using an initial screening form (Baseline only).

- d. Demographic information, personal and medical history, hormonal and reproductive history, exercise, smoking and alcohol use history obtained by an RD using the Epidemiological Questionnaire (Baseline only).
- e. Anthropometric measurements such as subject's height, weight, skinfold and circumference measurements (Baseline, week 6 and 12).
- f. 30 mL Blood samples will be drawn into heparinized tubes in a non-fasting state at the same time of day, between 7:00 AM and 12:00 PM, for each individual to perform hormonal assays and total and percentage free prostate specific antigens (Baseline, week 6 and 12). Hormonal assays will include free testosterone, sex-hormone binding globulin and estradiol at baseline, week 6 and at week 12. As there are no previous studies that have established the duration required to demonstrate change in hormonal levels with intake of genistein in males, we adopted the time taken to demonstrate hormonal changes with ingestion of genistein in female populations which is within one menstrual cycle. We had thus established the evaluation point as 12 weeks or 3 months for both the female and male groups. The hormonal assays (radioimmunoassay) will be performed by Quest Laboratories. Blood draws are done by the Cancer Center phlebotomist and processed and shipped using standard procedures for shipping to Corning Nichols, who will perform the radioimmunoassays.
- g. A biopsy and digital rectal exam will be performed by the GU program chief/oncologist for all patients entered in the study at baseline (routine), which will determine patient's admissibility to the study.
- h. The participant will be provided with a 2-day diet record (TDFR) and instructed on reporting food intake, including weights/measures and methods of preparation of foods consumed using standard food models (Baseline, weekly).
- i. Changes may be anticipated in stool frequency or GI discomfort. A pre-validated Nutritional Symptoms Scale is used to monitor GI symptoms during intake of supplements on a weekly basis.
- j. A Participant Tracking Form is used to monitor all activities and variables observed during the study period. Activities of each participant are vital for the study such as use of supplements, compliance to all monitors. This form will, in addition, serve as a checklist to monitor these variables for the Project Dietitians.
- k. Quality control procedures for data collection and entry are ongoing.
- l. Contact numbers were provided to patients.

Task 2: Abstraction of Medical Records Data

- a. We have continued to obtain patient disease related prognostic indicators from medical charts.
- b. Data entry and quality control procedures have been initiated.
- c. Follow-up interviews for data collection periods at mid-point and post completion of interventions have been completed for 11 subjects and is ongoing for the remaining 9.
- d. Weekly visits to the cancer center to obtain supplements and submission of monitoring instruments are ongoing.
- e. Shipping of completed patient's blood sample for hormonal assays and PSAs are ongoing.

REPORTABLE OUTCOMES

We have recruited twenty-two (22) eligible subjects for the genistein/prostate cancer pilot study and 11 subjects have completed the study and eight (8) are active. Two subjects dropped out of the study as they were unable to tolerate the taste of the product.

As hypothesized, we have observed an average increase of 6 points in the ratio of total to free PSA from baseline to post intervention in the experimental group in 40% of the subjects, while the rest showed a decrease of less than 2 or no change in the ratio. Increasing PSA ratio over 24 is more likely to be benign, while PSA below 19 indicates prostatic carcinoma. In addition, serum estradiol levels increased in all subjects. Sex-hormone binding globulin increased in 40% of the subjects while free testosterone decreased in 40% of the subjects in the experimental group. If increased intake of the isoflavone genistein alters the sex hormone concentration, bioavailability or metabolism, manipulation of the diet by adding rather than restricting food may reduce breast and prostate cancer risk.

CONCLUSIONS

As planned, we will continue to recruit a total of 66 subjects in the study, collect and complete data entry, analyze and report this data, testing the following hypothesis, as proposed.

1. Supplementation with the isoflavone genistein will produce an increase in serum levels of sex-hormone binding globulin (SHBG). This increase will exceed that of a placebo control group. A pooled t-test will compare the two groups' mean SHBG levels at the conclusion of Phase II.
2. Supplementation with the isoflavone genistein will produce a decrease in serum-free testosterone. These decreases will exceed that of a placebo control group. Pooled t-tests will compare the two groups' mean free testosterone levels at the conclusion of Phase II.
3. Supplementation with the isoflavone genistein will produce an increase in serum estradiol. This increase will exceed that of a placebo control group. A pooled t-test will compare the two groups' mean estradiol levels at the conclusion of Phase II.

Pooled t-tests will, in addition, be used to compare mean changes in intake of other nutrients, body composition parameters and nutritional symptoms at the end of Phase II. Pooled t-tests are justified in this case. Even if the data are only approximately normally distributed, the test is well known to be quite robust with respect to the normality assumption. These tests will be two-sided.

Multivariate repeated measures ANOVA will be performed on each variable with time as the repetition variable and treatment group as class variable. "Time effect", "group effect" and "time by group" interaction will be tested. If this last effect is significant, then "time effect" will only be reported separately for each group and "group effect" will only be reported separately for each time. The need to adjust for multiple testing is somewhat controversial. We will exercise some control over the multiple testing problem by comparing groups at each time point only if the overall ANOVA is significant. We recognize that this analysis may have limited power due to

missing data. For that reason the repeated measures ANOVA will be considered a secondary analysis and the methods described previously will be primary.

At the end of phase II, but before unblinding, patients will be asked whether they had experienced more GI distress than usual. The two groups will be compared with respect to the proportion answering affirmatively to this question using the Chi-square test for equality of proportions. A 95% confidence for the difference in these proportions will be constructed. The two groups will also be compared for the mean change in total PSA levels and mean percentage of free PSA using the pooled t-test. Ninety-five percent confidence intervals for the differences of these group means will also be formed.

**Phase IIa Chemoprevention Study of Selenium
in Persons at Risk for Lung Cancer
Principal Investigator: Gail L. Shaw, M.D.**

INTRODUCTION

Much knowledge has been acquired about the multi-step process of carcinogenesis in the lung during the last 20 years. Tumorigenesis appears to be the result of a number of genetic insults, although it remains to be determined whether there is a necessary sequence or a critical number of events required. Certain genetic alterations can be detected in the bronchial epithelium of persons at increased risk for lung cancer. Selenium may act through several different mechanisms of action, including stimulation of apoptosis, protection of tissue against oxidative damage, inhibition of tumor growth, reduction of mutagenic activity and reduction of activation of carcinogens and stimulation of the immune system. Selenized yeast has also recently been shown to reduce lung cancer incidence and mortality in a population of skin cancer patients. Smokers and survivors of early stage lung and head and neck cancers have had a long period of promotion by carcinogenic agents on the bronchial epithelium resulting in morphologic and molecular alterations. **We hypothesize that these morphologic and molecular alterations can be detected and modulated by chemopreventive agents.** We have proposed a Phase IIa chemoprevention trial evaluating five different dose levels of selenium administered daily for 3 months in subjects at high risk for lung cancer with bronchoscopically documented dysplasia. After establishing the maximum tolerated dose, additional subjects will be entered at that dose level in order to examine the modulation of biomarkers in response to selenium supplementation as well as to measure selenium levels and modulation of glutathione peroxidase as a measure of drug effect. In addition to morphology, the surrogate endpoint biomarkers to be examined include apoptosis, p53 expression, K-ras mutation analysis, p16 methylation, and upregulation of hnRNP A2/B1. Successful completion of this study will support selenium supplementation as potentially beneficial therapy in preventing the progression of lung carcinogenesis as well as identify surrogate endpoint markers that appear to be modulated by selenium supplementation.

BODY

The Statement of Work was submitted as a timeline. Initial patient accrual refers to the enrollment of subjects on the dose-finding portion of the study, and this is still currently underway. No secondary patient accrual has begun, as completion of the first phase is required before continuing on to the second phase.

Since DOD approval 25 March 1999, 17 subjects have consented to participation and undergone sputum induction. Eight were eligible to proceed to bronchoscopy and 7 of these have been completed. Six of the 7 had documented bronchial dysplasia and have proceeded to receive the selenized yeast in a randomized fashion, as described in the protocol. Four subjects remain on the supplement.

Since the study was originally initiated, a total of 492 subjects have been screened for the study, with 155 eligible to participate. Of those eligible, 147 chose to participate in the study. Sputum inductions have been performed on 146 individuals. Thirty-seven were normal, 99 showed some

Data is presented by smoking and bronchoscopy finding in Figures 1-3.

Selenium Blood Levels

Blood samples taken from 9 selenium participants at baseline and 3 months show increase in blood selenium levels for all subjects. Average selenium level at baseline was 124.4 mcg/l and at 3 months was 292.8 mcg/l (Figure 4).

Adverse Events

Six adverse event reports were filed, none of which were determined to be study related. A summary of the events is shown below.

Patient ID	Date of Event	Event	Study Related	Outcome
003	5/01/98	Asthma	No	Resolved
010	8/18/98	Cough, chest tightness, fever	No	Resolved
010	8/25/98	Follow-up	No	Resolved
074	12/17/98	Vaso-vagal response, post bronchoscopy	Possibly	Resolved
014	1/10/99	Hernia	No	Herniorrhaphy
014	2/01/99	Fever	No	Resolved

No toxicities developed for any dose level of selenium.

Laboratory Progress Report

For the ten individuals who have completed 3 months of selenium supplementation on study:

- 118 biopsies, formalin fixed and paraffin embedded.
- 20 blood samples, with glutathione peroxidase (GPX) assays done in triplicate including control assays for no substrate, no sample and a positive control with purified GPX. All 20 samples have been retested with an improved protocol and compared for variation (Figures 5-7).
- Methylation specific PCR of the p16 gene has been done on both the baseline selenium-sputum samples. No aberrant methylation patterns have been detected (Figure 8).
- 15 biopsy blocks have been sectioned to exhaustion in preparation for microdissection.

For all study subjects

- Tissue culture of normal bronchial epithelial cells (NHBE)
 - 50 study subjects
 - 246 NHBE cultures, 135 grew enough to be propagated in a second flask.
 - Culture failures to contamination were 3.6% in the P0 (9/246) and 8.9% in P1 (12/135).
 - Culture failures to insufficient numbers of cells were 28 % in P0 (69/246) and 4.4 % in P1 (6/135).

- Failed cultures are defined as those that did not produce any colonies after one month in culture.
- Most cultures have cells stored for extraction of DNA. A few cultures judged to be sufficiently proliferative were prepared for cryostorage in liquid nitrogen.
- DNA has been prepared from either the cells grown in culture or from cells that failed to attach to the culture dish.
- All cultures that could be cultivated will have cells stored for the extraction of DNA.
- Blood samples
 - 50 blood samples processed for isolation of lymphocytes and for the baseline measurement of GPX.
 - For individuals who have received selenized yeast, an additional 10 blood samples have been processed for isolation of lymphocytes and measurement of GPX.
 - All samples: whole blood and lymphocytes, are stored at -80°C.
- Biopsies
 - All biopsy blocks are stored and controlled by the Pathology Department of the H. Lee Moffitt Cancer Center. In general, for most study subjects the number of biopsies is the same as the number of sites used for collecting NHBE for cell culture. For a few study subjects, extra biopsies were taken at the discretion of the physician. Comparison interpretation by a blinded consultant has been completed and those interpretations are currently being compared with those by our study pathologist.

KEY RESEARCH ACCOMPLISHMENTS

- Established feasibility of recruiting and enrolling heavy current and former smokers on a chemoprevention study.
- Developed algorithm to recruit and screen subjects, obtain induced sputum specimens, obtain history and physical and screening chest x-ray and blood work prior to bronchoscopy, obtain bronchoscopy and start eligible subjects on selenium supplement.
- Developed close collaborative relationships with pulmonary medicine and pathology.
- Evaluated induced sputa from high risk individuals for p16 hypermethylation (all with no hypermethylation detected).
- Developed archive of 246 bronchial epithelial cell cultures.
- Developed archive of induced sputum specimens from 146 high risk individuals.
- Evaluated the value of fluorescent bronchoscopy in addition to white light bronchoscopy in predicting dysplasia in a high risk population.
- Measured glutathione peroxidase pre- and post-selenium supplementation and found no change.
- Measured selenium pre- and post-selenium supplementation and found increase (dose still blinded).

REPORTABLE OUTCOMES

- Shaw, G.L., Walsh, F.W., Rolfe, M.W., Cantor, A.B., Vann, V., Khor, A. Value of fluorescent bronchoscopy in identifying bronchial metaplasia and dysplasia in smokers. *Proc. Annu. Meet. Amer. Soc. Clin. Oncol.* 18:476a, (abstract 1836), 1999.
- Development of repository of induced sputum specimens from 146 individuals.
- Development of repository of bronchial epithelial cell cultures (246 cultures from 50 study subjects).
- Serum and lymphocyte repository from the same patient population.

Figure Legends

Figure 1: Histopathology diagnosis correlated with smoking status. Multiple biopsies were taken for each individual. The frequency of the most severe bronchial histopathology is correlated with the smoking status obtained from the enrollment questionnaire.

Figure 2: Histopathology diagnosis correlated with the observed white light bronchoscopy. Multiple biopsies were taken for each individual and each biopsy has both the histopathology diagnosis as well as the interpretation by white light (White light classification is: Class I is normal; Class II is characterized by inflammation and/or metaplasia to mild atypia; Class III is characterized by moderate to severe atypia).

Figure 3: Histopathology diagnosis correlated with the observed fluorescent light bronchoscopy. Multiple biopsies were taken for each individual and each biopsy has both the histopathology diagnosis as well as the interpretation by white light (Fluorescent light classification is: Class I is normal; Class II is characterized by inflammation and/or metaplasia to mild atypia; Class III is characterized by moderate to severe atypia).

Figure 4: Blood selenium levels. Levels of elemental selenium were measured at baseline and after taking selenomethionine supplementation for 3 months. Blood samples were collected in trace element collection tubes and selenium levels were measured by atomic absorption spectroscopy (SmithKline Beecham).

Figure 5: Glutathione peroxidase (GPX) activity in red blood cells. The assay was done in triplicate for each blood sample collected at baseline and after 3 months of selenomethionine supplementation. A separate assay with purified GPX was done as a positive control and assays without sample or the organic peroxide substrate were done as negative controls. The assay is an indirect measure of GPX activity where oxidized glutathione is produced upon reduction of organic peroxide (*tert*-butyl hydroperoxide) and is recycled to its reduced state by exogenously added glutathione reductase (GR). The reduction of oxidized glutathione by GR requires the oxidation of NADPH. The GPX activity is determined by a decrease in absorption at 340 nm as NADPH is oxidized to NADP. Using the molar extinction coefficient for NADPH ($6220 \text{ M}^{-1}\text{cm}^{-1}$) and the rate of decrease in absorbance at 340 nm, the GPX activity for each sample can be calculated.

Figure 6: Hemoglobin measurements at baseline and after 3 months of selenomethionine supplementation. Total hemoglobin was measured from lysed red blood cells using a kit purchased from Sigma Diagnostics (Cat. No. 525-A). The procedure is based on the oxidation of hemoglobin to methemoglobin and the subsequent conversion of methemoglobin to cyanmethemoglobin by potassium cyanide which has a maximum absorption at 540 nm. The color intensity measured at 540 nm is proportional to the total hemoglobin concentration. Note that hemoglobin values measured in this way are higher than those obtained with other clinical chemistries.

Figure 7: Glutathione peroxidase activity per gram hemoglobin in blood samples taken at baseline and after 3 months of selenomethionine supplementation. See Figures 5 and 6 for details of the methods.

Figure 8: Methylation specific PCR on DNA sputum samples. The upper panel shows the PCR products on DNA from baseline sputum samples using primers specific for unmethylated DNA. The lower panel uses the same DNA but with primers specific for methylated DNA. Note that most of the cells in a sputum sample should be normal and thus most sputum samples should produce PCR products representing unmethylated (normal) p16 gene. DNA was extracted from cells in the sputum samples collected at baseline and after selenomethionine supplementation by standard proteinase K/SDS digestion followed by phenol extraction and ethanol precipitation of the DNA. The DNA is treated to convert all cytosine bases to uracil but methylated cytosine is not converted. After purification, this DNA is used for PCR amplification of a region of exon 1 of the p16 gene known to be susceptible to hypermethylation. Hypermethylation has been shown to repress transcription of the nearby gene. Specific PCR primers are designed to recognize the modified DNA if the DNA is either methylated or non-methylated. Separate PCR reactions are done with the specific primers to determine the methylation status of the region. The PCR products are evaluated by agarose gel electrophoresis. Positive controls from cell lines are used to show specificity of the reaction. The Calu-6 cell line contains a hypermethylated exon 1 for the p16 gene while the p16 gene for the Calu-3 cell line is not hypermethylated.

Histopathology

Most Severe Bronchial Histopathology by Smoking Status

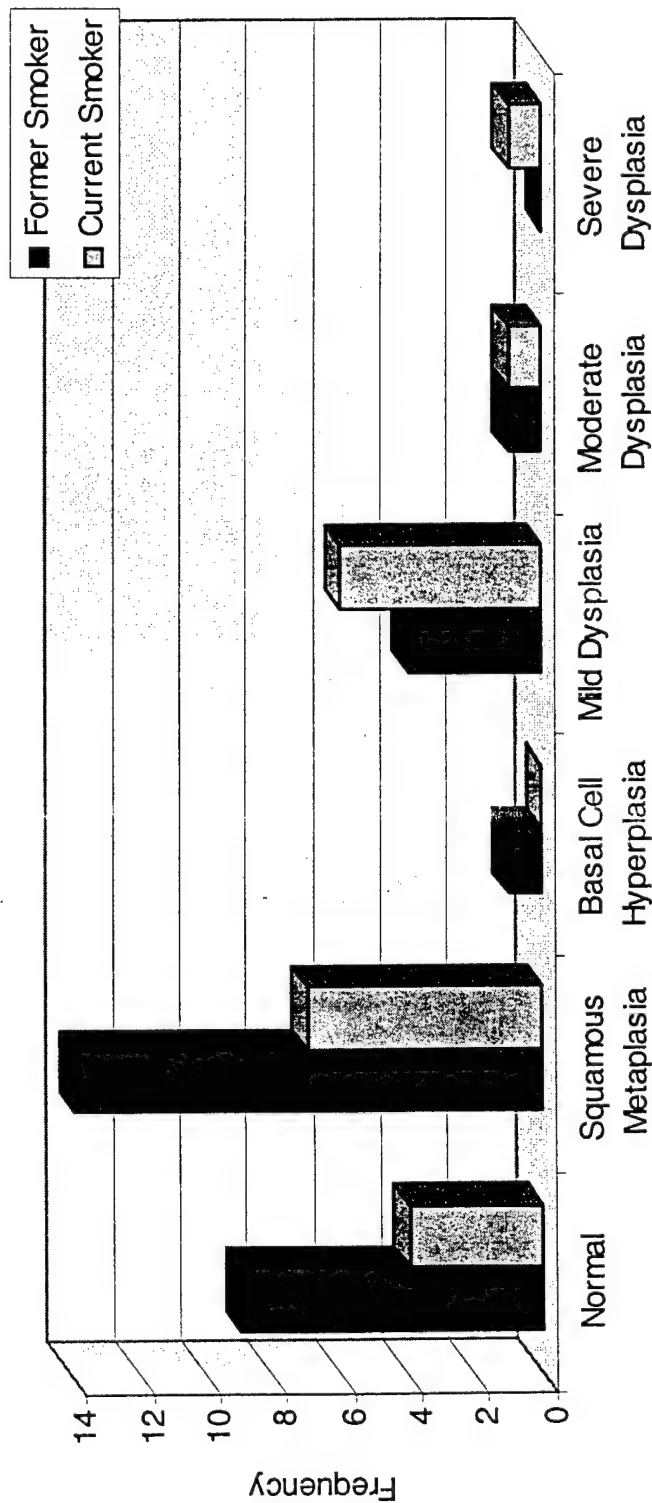


Figure 1

Histopathology

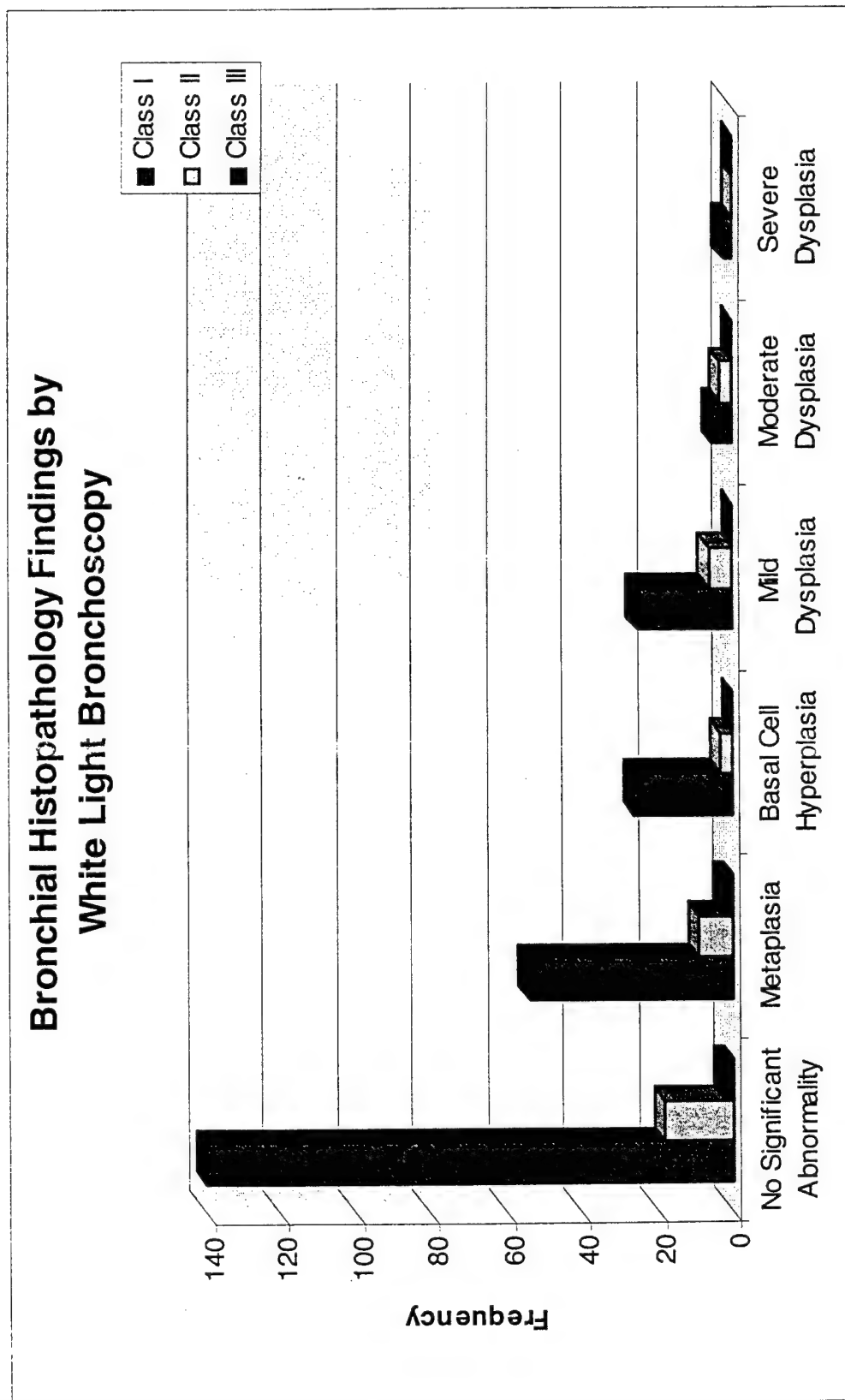


Figure 2

Histopathology

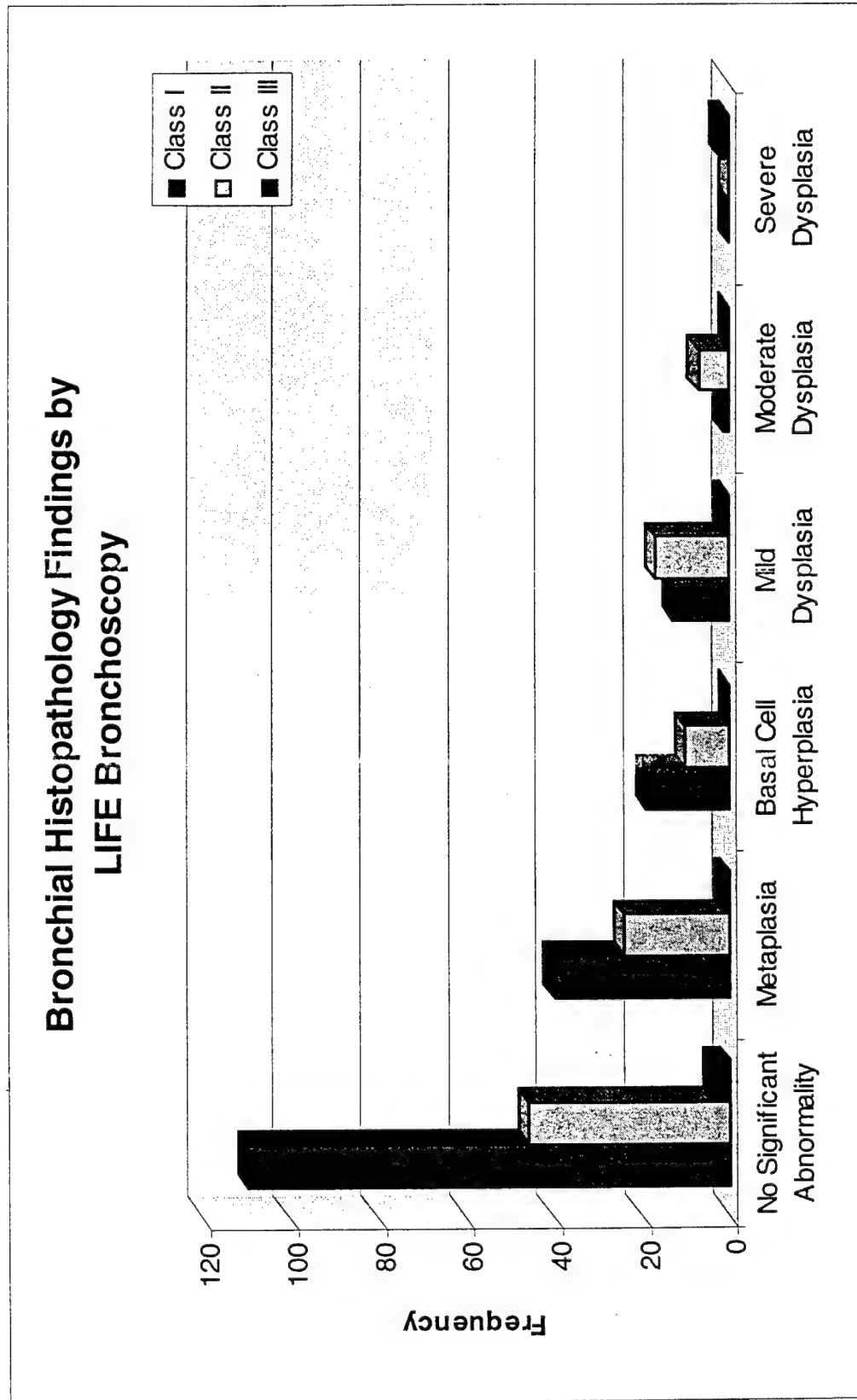


Figure 3

Blood Selenium Levels at Baseline and 3 Months

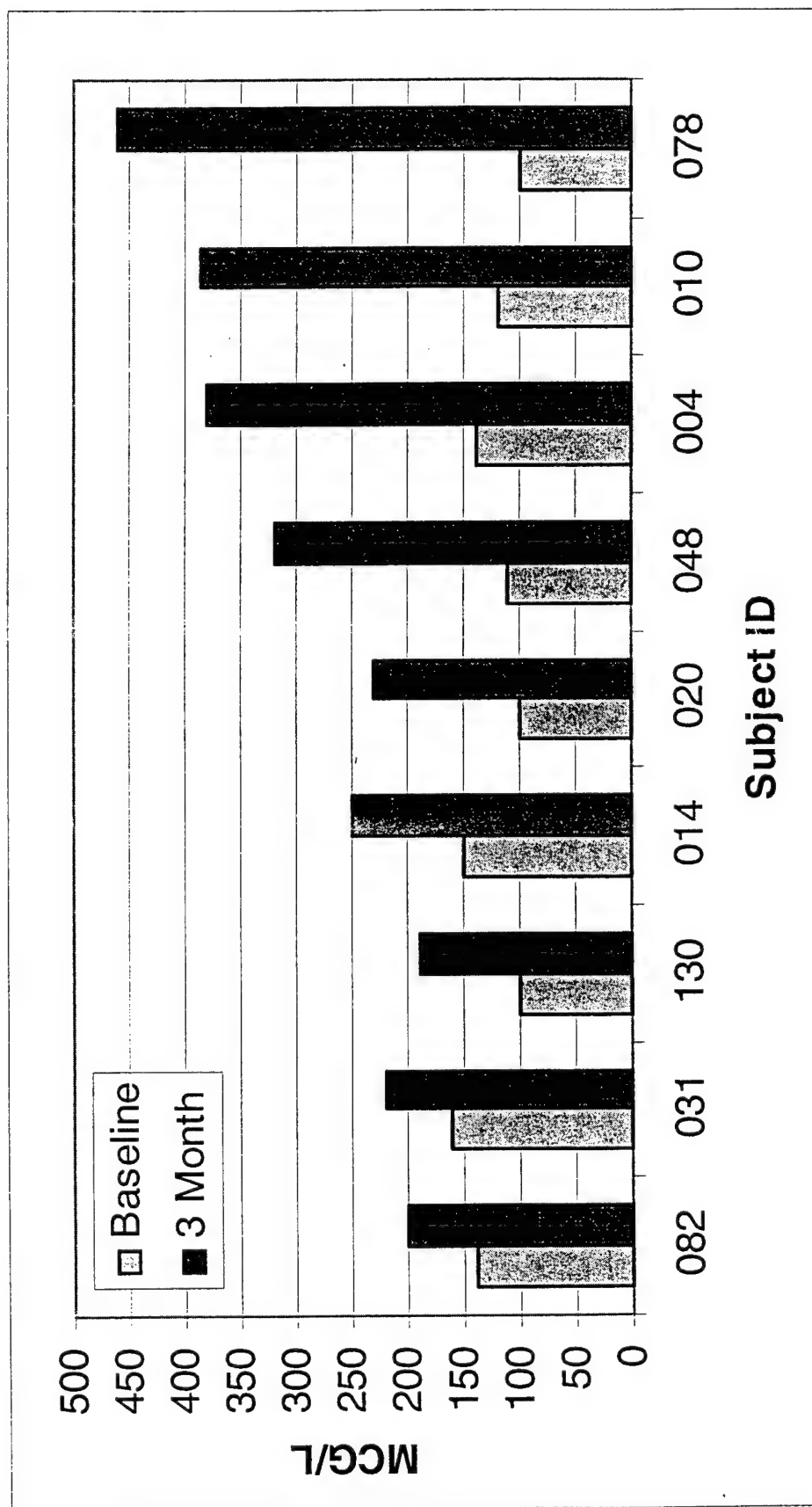


Figure 4

Glutathione Peroxidase Activity at Baseline and 3 Months

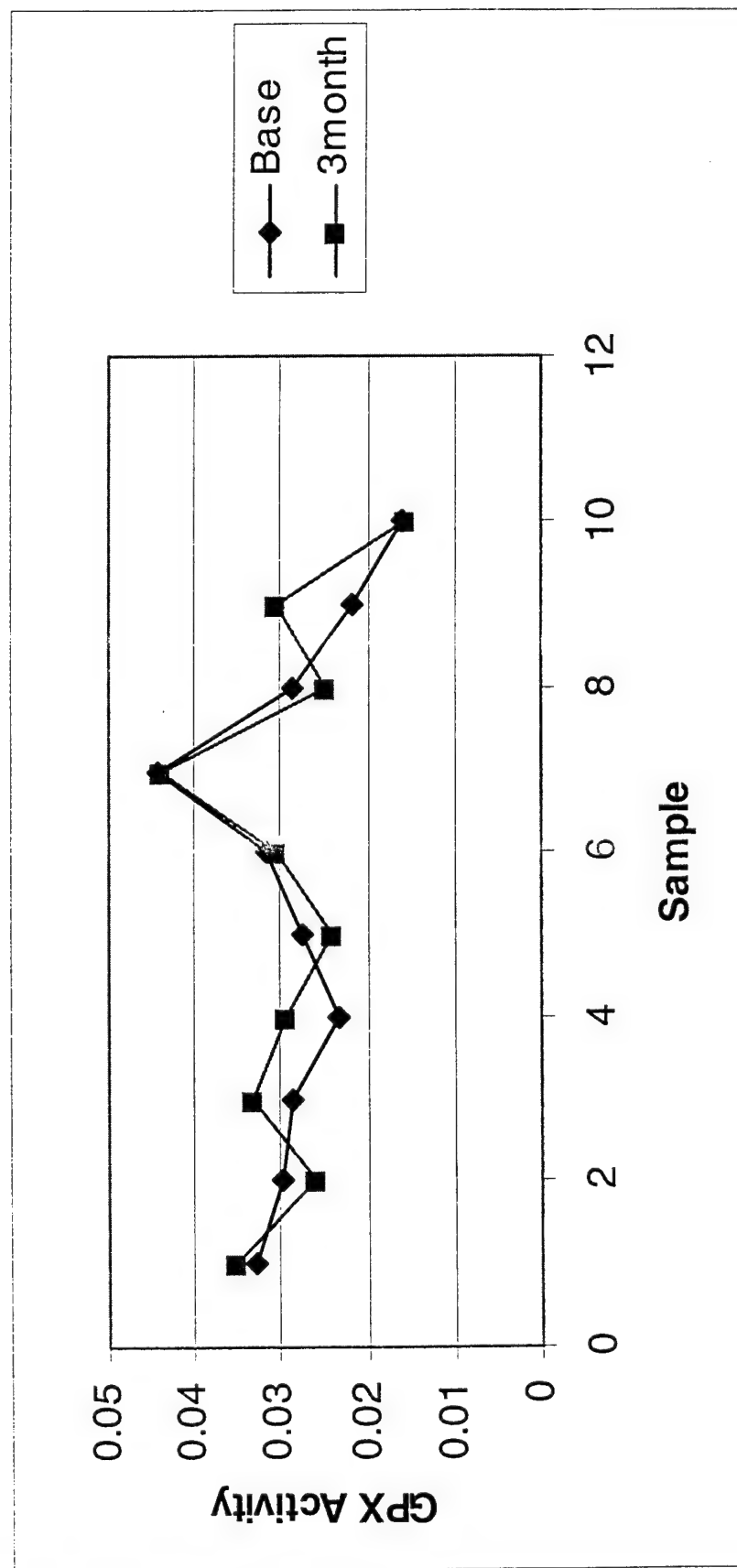


Figure 5

Hemoglobin at Baseline and 3 Months

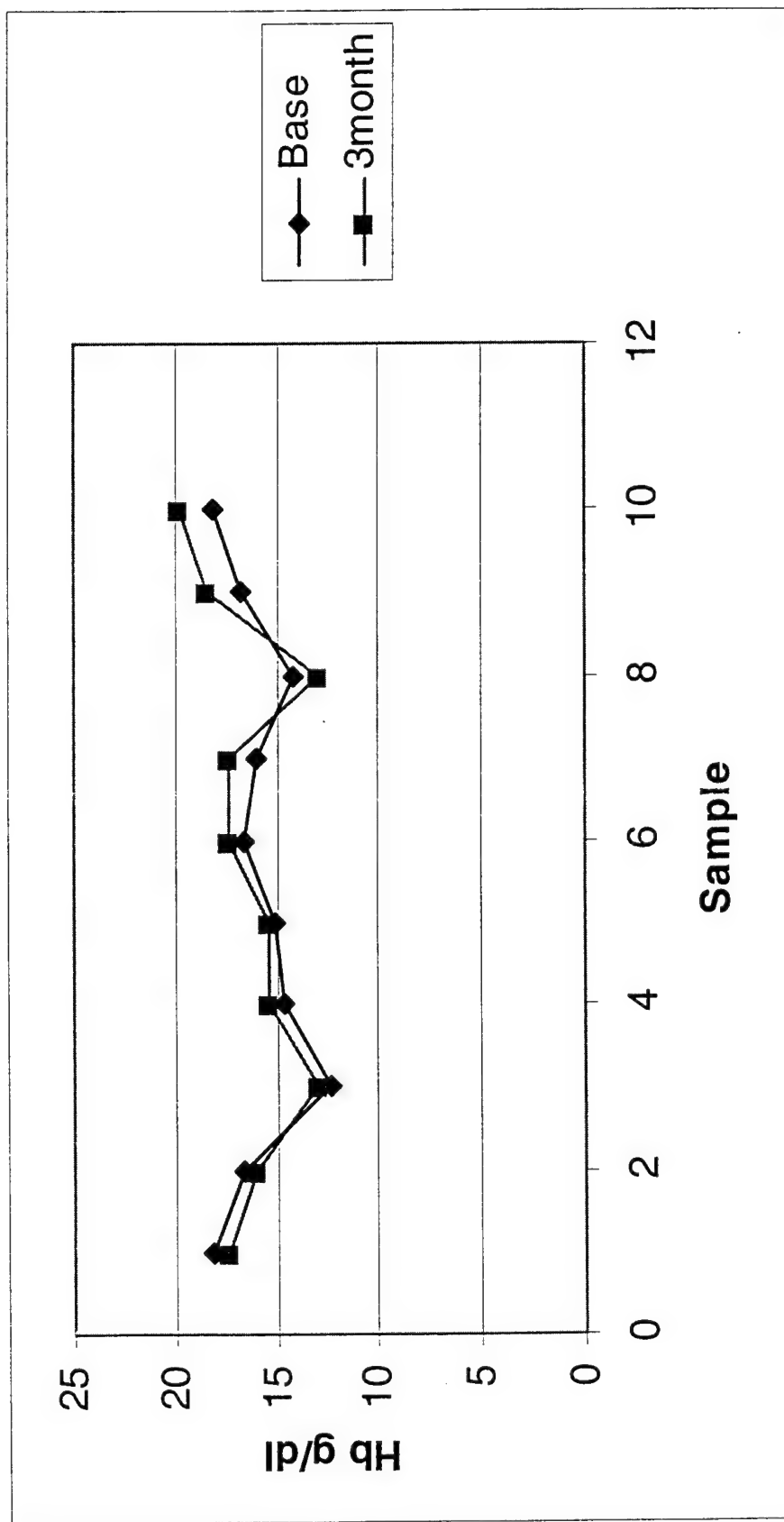


Figure 6



GPX Activity / g Hemoglobin at Baseline and 3 Months

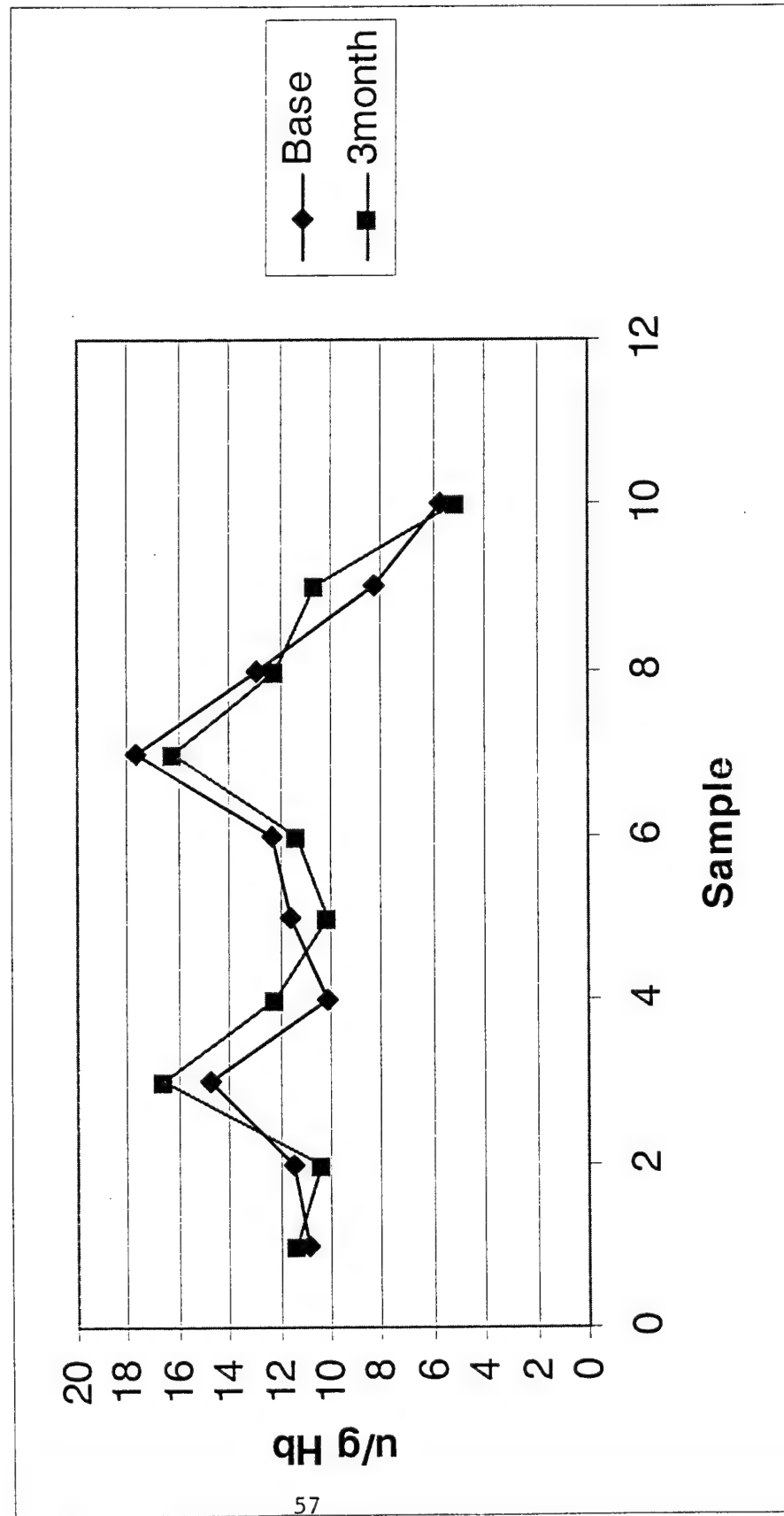


Figure 7

MSP on p16 using DNA from Baseline Sputum

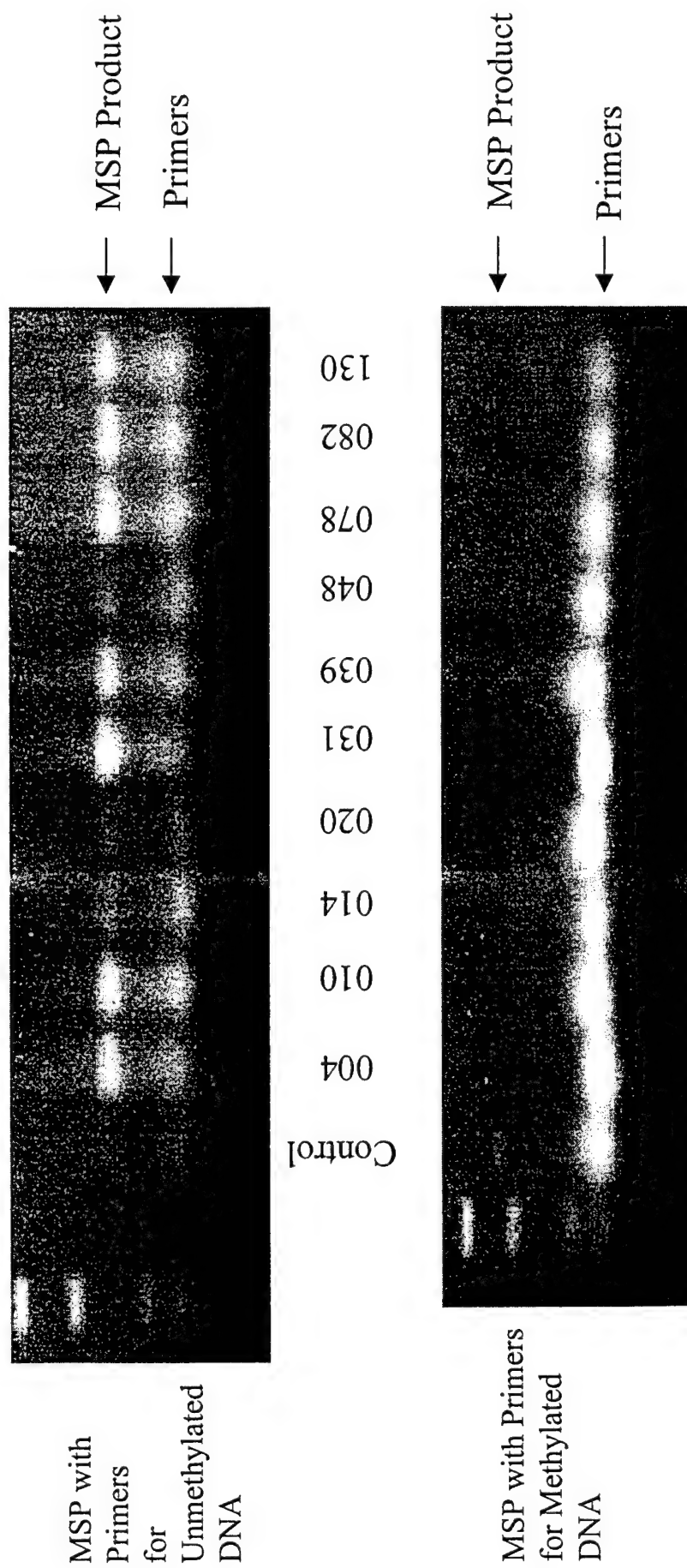


Figure 8

Development of the Moffitt Cancer Network as a Telemedicine and a Teleconferencing Educational Tool for Health Care Providers

Principal Investigator: Jeffrey P. Krischer, Ph.D.

INTRODUCTION

The Moffitt Cancer Network's (MCN) goal is to provide up-to-date oncology related information, resources, and education to oncology health care providers and researchers for the prevention and cure of cancer. Consistent with the aims of the Advanced Cancer Detection Center, the MCN provides access to educational programming, cancer control and clinical protocols, and a mechanism to exchange patient focused information leading to the improved detection and treatment of cancer. The MCN is health care provider focused and complements an array of existing public/lay information sources available elsewhere. It is built around the concept that oncology expertise is geographically centralized, multidisciplinary in nature and of limited availability. The MCN addresses these constraints by increasing availability through a World Wide Web-based design that enables wide access from many geographic locales. The objectives of this project are to 1) collect and organize cancer information to provide educational content to physicians and other health care providers, 2) develop and implement software to encode video and audio to enable viewing over the Internet at a range of speeds (bandwidths), 3) implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation, 4) design and create a web page to permit easy sorting, searching and selection of educational programming, 5) design and create a web page to deliver physician referral information that includes submission of an electronic case record consisting of text and imaging data, and 6) provide access to case conferencing from remote locations using easily available audio/video to the desktop.

ACCOMPLISHMENTS

The MCN uses a software product called 'TAG Composer', produced by Digital Renaissance, to assemble the images and audio together into a streaming format, synchronized by using a set of timings. The significance of this very new software (TAG Composer) is that it produces a new kind of streaming format (Sure Stream) that is deliverable over the Internet regardless of bandwidth. This new streaming format is different from traditional streaming 'video' format. A Sure Stream format contains within it the intelligence to respond transparently to a variety of bandwidth situations. Indeed it accommodates a 'changing' bandwidth scenario, dynamically adjusting buffering algorithms to adapt to network congestion (or the lack of it).

The MCN is constructed as a set of channels of information. The educational offerings are organized as the Virtual Classroom Channel and the physician referral/consultation functionality is termed the Physician Referral Channel. The Virtual Classroom Channel provides a range of educational opportunities for authorized users. Primary content is provided by Moffitt sponsored Grand Rounds, and presentations made by faculty at national scientific meetings. The Physician Referral Channel provides access to authorized users to a) search Moffitt open clinical protocols to enhance a patient's care, b) send on-line patient information about a patient, use videoconferencing technology to consult, in real time, with Moffitt physicians about patient care

issues and c) use an on-line search tool to gain access to clinical information about their patients who receive some part of their care at the Moffitt Cancer Center.

Accomplishments specific to the approved Statement of Work are as follows:

Task 1. Collect and organize cancer information to provide educational content to physicians and other health care providers. (Months 1-60)

A schedule of events is compiled by the Network Coordinator from information received from the Moffitt Department of Education, individual research areas who sponsor guest speakers, and Moffitt and USF offices of Conference Planning. This schedule is given to the MCN videographer who provides video and audio capture of presentations. The schedule usually consists of an average of 15 to 20 hours to be captured over a one to two month period. The decision to capture a presentation is based on quality of the speaker, content and topic timeliness for the audience of health care providers. A major goal of the MCN is to provide Continuing Medical Education (CME) or Continuing Educational Units (CEU) for all presentations. The network currently consists of 81 total presentations. Of these, 35 presentations are representative of six conferences sponsored by USF and Moffitt and guided by USF faculty who hold major responsibilities at Moffitt (i.e., program leaders, department heads, etc.).

Task 2. Develop and implement software to encode video and audio to enable viewing over the Internet at a range of speeds (bandwidths). (Months 1-60)

The technology used in the TAG software provides a streaming picture video of a presenter's slides while their entire audio presentation is carefully synchronized to the slides. Thus, a user who accesses a presentation sees just what a meeting attendee sees, but with an added advantage. Using the RealPlayer viewer, the computer user is able to pause the presentation at any point to look closer at slides or accommodate an interruption, and can rewind the presentation to hear and/or see again a particular part of the speaker's talk. The server employed to access videos on the Network provides up to 100 concurrent viewers of the same video with no loss of quality. A secure screen is provided for payment of subscriptions as well as payment for continuing education credits. Authorized use is provided free of charge to Moffitt employees, staff members of the two area VA hospitals, as well as health care professionals at the MacDill Air Force Base.

Task 3. Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation. (Months 1-60)

User information is collected on a registration form which is submitted to the Network database. This information is used for tracking access to the Network by each user. Each presentation that provides CME or CEU is encoded with three alphanumeric codes which a viewer must record and submit in order to receive credit (see appendix for example). These codes must be entered exactly as they appear at random intervals throughout the presentation, thus ensuring that the user has, indeed, viewed the entire presentation. Once the codes are submitted and checked by the computer, the viewer's information is forwarded to the University of South Florida Office of Continuing Medical Education for issuance of a certificate via mail.

The Cancer Center Library is automatically linked to the acquisition process so that they are aware of new acquisitions and extract key words for indexing, sorting and searching. Of the 81

total presentations available on the Network as of this report, 20 are available for CEU's for nurses. Meetings are presently underway with Moffitt's Pharmacy Department to develop offerings for pharmacy personnel. Included in the Network offerings will be JCAHO requirements for risk analysis, HIV, infection control, etc.

The Network Coordinator is automatically notified via e-mail by Department of Education staff of each new grand rounds presentation, via interoffice mail by sponsoring offices of each sponsored seminar, and through the conference literature by Conference Planning staff for conferences to be held off-site. The Network Coordinator then notifies Department of Education staff, Conference Planning staff, or sponsoring office staff of presentations selected for the Moffitt Cancer Network through phone call, e-mail or meeting, as needed. Presenters of grand rounds are asked by Education staff to sign a release form prior to taping. Presenters of special seminars are given the release by a member of the sponsoring office's staff. Presenters at conferences are approached by a member of either the Education Department or Conference Planning.

Task 4. Design and create a web page to permit easy sorting, searching and selection of educational programming. (Months 1-24)

Information on each presentation is recorded in a database using the Microsoft Access program. As noted in Task 3, the Cancer Center Library automatically receives an e-mail letting them know when a presentation is ready to have key words assigned. The librarian views each presentation and assigns key words according to a standard nomenclature utilizing NLM MeSH headings, cancer site, etc. In addition, the database contains a field for choosing the audience for each presentation (physician, nurse, etc.). These fields enable a user to search for key words or sort videos by audience. Utilization is monitored and reports prepared to enable the Network staff to better define and target future offerings for users.

Task 5. Design and create a web page to deliver physician referral information that includes submission electronic case record consisting of text and imaging data. (Months 1-36)

Oracle8i software will be used to implement a database to archive text and imaging data for retrieval by consulting Cancer Center physicians and integration with Moffitt Cancer Center clinical information systems. Oracle8i can store complex image data as well as user defined objects. Since the institution already uses Oracle8i as the backend for all clinical systems, this will facilitate integrating Physician Referral with existing clinical systems.

We are researching using the Health Level 7's Message Information Model to develop a structured computerized clinical case description that provides a minimally relevant set of data that describes a clinical case for second opinion and consultation. XML and Java are being evaluated for implementing HL7's message information model. This would provide a common data model across all the heterogeneous systems that Physician Referral would access because the Oracle8i supports both Java and XML, and both of these technologies are available for all the major computing platforms in the industry.

The Network uses Real G2 server to stream live as well as prerecorded presentations over the Internet. The Real G2 server receives its input from Real Producer Plus which is a software package that encodes any type of multimedia material into a format which the Real server can

stream out over the Internet. The encoder receives its input from Pinnacle Systems miroMOTION DC30 plus video capture card. The miroMOTION DC30 plus is a professional tool for creating high-quality video and audio productions and also provides video conferencing capabilities. The video card can obtain input from a number of sources: VCR, digital cameras, camcorders, etc.

The secure communications links for streaming presentations are provided by the Real server and Microsoft's Internet Information server. The web server provides secure 128-bit encryption and the Real server provides user level password security as well as presentation level security.

Due to the ubiquity of telephone connections in the home, and the LAN connections at businesses, the Internet is the most appropriate means for connections to multi-bandwidth communications. The encoder software that feeds the Real Server can encode multi-bandwidth streams so that the Real server can deliver them over the Internet.

We are researching the use of Java Applets and XML to construct the web frontends that would communicate with CERNER (Moffitt's patient information system). The Java Security model with IIS encryption would give us the means by which we are able to provide secure access to patient information as well as the submission of case conferences.

Task 6. Provide access to case conferencing from remote locations using easily available audio/video to the desktop. (Months 1-48)

The telegenetics project is awaiting IRB approval from the DOD. Once approval has been received, everything is in place to begin assessing feasibility and acceptability of this format for the exchange of clinical information.

The installation of the Checkpoint firewall software will resolve communication issues between Moffitt and remote sites. Once the software is installed (on November 3, 1999), we will be able to use the Internet, the Real server and IIS for communications between Moffitt and remote sites.

We are researching the use of a portable video unit with a built-in web server to facilitate the presence of technology in conference centers where case conferencing generally occurs. The unit is the Polycom video conferencing unit. It allows us to take video and audio from anywhere in Moffitt where there is a network connection and send it to the Real server for streaming out over the network. The unit allows the sharing of images, applications, documents, etc., with other institutions or users right over the Internet. This will permit selected clinics to retrieve and display multiple images and clinical data submitted for this purpose by remote users.

We are constantly assessing utilization of Network technology to refine and revise formats and improve the quality and ease of remote access.

KEY RESEARCH ACCOMPLISHMENTS

- Established and refined a system to capture high quality video and audio presentations.
- Developed database to allow for searching by key word or sorting by audience.
- Implemented video access over a variety of bandwidths.

- Implemented a mechanism to provide continuing education credits on-line.
- Provide free access to James A. Haley VA Hospital, Bay Pines VA Hospital, and MacDill Air Force Base.
- Provided secure communication links.
- Monitor utilization and procedures for access to ensure timely presentations as well as ease of use.
- Provided tour of features available.

REPORTABLE OUTCOMES

Several databases are maintained in relation to the Network. One contains user information obtained upon registration as a subscriber. This database will allow the reporting of frequency of access, length of each session, which presentations have been accessed, continuing education credits sought, and, eventually, whether the user accessed the presentation in English or Spanish. (Our plan is to provide audio in Spanish for all presentations.)

Another database contains information on the presentations themselves. Recorded there are the presentation title, presenter's name and affiliation, length of the presentation, target audience, whether or not continuing education credits are available, key words, and cancer site. This database "drives" what appears on the Internet, enabling searches and sorting as well as viewing presentations themselves.

A third database contains the alphanumeric codes assigned to each presentation for continuing education credits. Recorded here are the name of each presentation, the codes, and where (at what time mark) they are inserted into the presentation. Once a user has viewed a presentation, they submit their codes which are checked against this database. If all the codes are correct, then their information is forwarded to the USF Department of Continuing Medical Education where a paper certificate is issued and mailed to the user.

CONCLUSIONS

Great advances have been made on this project in the last year. Equipment purchased and employees skilled in the process of capturing presentations, digitizing the video and reuniting it with the audio have cut processing time in half. The addition of the manager and coordinator have brought a cohesiveness to the project which provides direction and constant quality control to ensure a state-of-the-art network offering the highest quality available.

In a review of health care related web sites, the coordinator examined 30 distinct offerings and performed an analysis of each. No other institutions (public or private) are offering the level and quality of services to be found on the Moffitt Cancer Network. Only our network provides the audio/visual presentation in such a way that slides can be viewed in a readable format. One other site offers a streaming video of presentations but the presenter is always viewed together with the slide. This presentation takes place using RealPlayer (the same software we use), however, the screen is less than half the size, rendering the sides virtually unreadable. Other sites which offer the slides in a readable format present the audio portion of the presentation as typed text which the user must read, therefore, absorbing more of their time. Likewise, ours is the only

presentation system presently using the alphanumeric codes to provide continuing education credits.

Advances in technology will continue to change the Network for the better as new items become available. Our goal is to remain cutting edge, constantly offering new presentations for users to access. We have targeted nursing as an area in which to increase the number of offerings and have placed a major emphasis on pharmacy as the next area to grow for the Network. As a major regional referral center, Moffitt will now be able to provide rural caregivers with access to physician consultations and patient follow-up via the Network, thereby increasing (or providing for the first time) access to world class physicians specially trained to deal with cancer cases.

APPENDICES

- Screens from the Moffitt Cancer Network:
 - Opening screen from the MCN tour
 - User registration screen
 - List of conferences available for view
 - Example of individual presentation from a conference
 - Example of individual presentation which offers CME and CEU
 - CME Verification screen
- Listing of equipment and software used for various aspects of operation
- Directions for assembling assets

Opening screen from the Moffitt Cancer Network Tour

The Moffitt Cancer Network (MCN) provides information, resources, and education to oncology healthcare providers and researchers. The MCN also makes available a secure mechanism to exchange patient focused information leading to the improved detection and treatment of cancer.

MCN consists of the *Virtual Classroom* and *Physician Referral*. The *Virtual Classroom* offers continuing education credits by accessing a wide range of cancer related topics as both individual presentations and full conferences. *Physician Referral* will provide access to Moffitt open clinical protocols. It will also provide the ability to exchange patient information and use video conferencing to consult with Moffitt physicians.



Registration

The information you will provide on this form is necessary to insure that you get proper CME or CEU credit for watching the presentations within MCN. Please fill in all the fields below. You will be asked to choose a user ID at least four characters long and a password. Choose something that will be easy for you to remember and write them down. Your user ID and password will be case sensitive and you will need them to gain access to everything MCN has to offer. After completing this form click on the "Next" button.

User ID:

Password:

Re-enter Password:

>> please do not use dashes or slashes <<

PERSONAL DETAILS

Social Security #:

>> all fields are required <<

Title (MD, ARNP, PA...):

Last Name:

First Name:

Email address:

Street Address:

City:

State:

Zip:

Country:

Phone Number:

Fax:

<< if available

PROFESSIONAL DETAILS

☐ Physician

☐ Nurse

☐ Other

Specialty:

- ☐ Medical Oncology/Hematology
- ☐ Radiation Oncology
- ☐ Pediatric Hematology/Oncology
- ☐ Infectious Disease
- ☐ OB/GYN
- ☐ Oncology Pharmacy
- ☐ Administration
- ☐ Oncologic Basic Science

- ☐ Surgical Oncology
- ☐ Gynecologic Oncology
- ☐ Immunology
- ☐ Urology
- ☐ General Surgery
- ☐ Oncology Nursing
- ☐ Psychosocial Oncology
- ☐ Other

Next >>

Conferences

Date: 9/23/99

Cancer Communications - Talk, Tools & Technology: (Presentation 1 of 6) Future Directions in Cancer Patient Education - Using New Media to Enhance Cancer Communications

Speaker: Barbara K. Rimer, Ph.D.
National Cancer Institute

Date: 9/24/99

Cancer Communications - Talk, Tools & Technology: (Presentation 2 of 6) Cancer Patient Education - Socio-Cultural Perspectives

Speaker: Roberta Baer, Ph.D.
University of South Florida

Date: 9/24/99

Cancer Communications - Talk, Tools & Technology: (Presentation 3 of 6) Getting in Touch with the Technological Side of Patient Education - Responsibilities, Ethics & Innovations

Speaker: John Groczik, M.A.
University of Wisconsin-Milwaukee

Date: 9/24/99

Cancer Communications - Talk, Tools & Technology: (presentation 4 of 6) Evaluation of Cancer Related Websites on the WWW

Speaker: Kelli McCormack-Brown, Ph.D., C.H.E.S.
University of South Florida

Date: 9/24/99

Cancer Communications - Talk, Tools & Technology: (presentation 5 of 6) Creating Relevant Patient Education Tools

Speaker: Louise Villejo, M.P.H., C.H.E.S.
M.D. Anderson Cancer Center

Date: 9/24/99

Cancer Communications - Talk, Tools & Technology: (presentation 6 of 6) Effective Caregiving and End-of-Life Communications

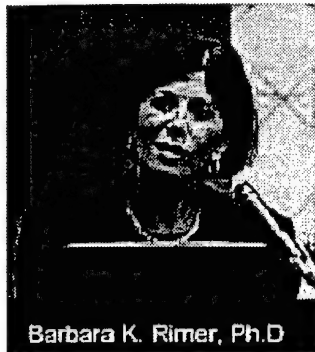
Speaker: Michael Weitzner, M.D.
H. Lee Moffitt Cancer Center & Research Institute

Presentation Length : 33 Minutes

Cancer Communications - Talk, Tools & Technology: (Presentation 1 of 6) Future
Directions in Cancer Patient Education - Using New Media to Enhance Cancer
Communications

Date: September 23, 1999

Presenter: Barbara K. Rimer, Ph.D.



Director
Division of Cancer Control and Population Sciences
National Cancer Institute
Bethesda, MD

Objectives: After viewing this presentation, you will be able to:

1. Discuss how new media can be used to support and enhance cancer communications.
2. Discuss what types of new technologies work best.

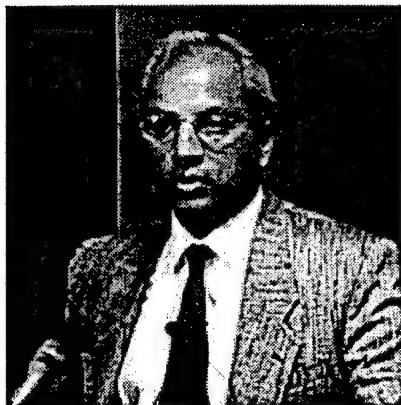
View Presentation

Presentation Length : 42 Minutes

Viral Hepatitis and Hepatocellular Carcinoma: Current Status

Date: July 30, 1999

Presenter: K. Rajender Reddy, M.D.



Professor of Medicine
Division of Hepatology
University of Miami School of Medicine
Miami, FL

Objectives: After viewing this presentation, you will be able to:

1. List predisposing conditions for HCC.
2. Analyze factors determining the cost-effectiveness of screening.
3. Cite clinical, laboratory, radiographic and histological results utilized in the diagnosis of HCC.
4. Evaluate various treatment options for HCC.

Supported through an unrestricted educational grant from Schering Oncology/Biotech.

View Presentation

View Presentation for CME

View Presentation for CEU

Verification



During the presentation, you will be shown three alphanumeric codes. As you see each one write it down exactly as it appears in the presentation. After you have watched the entire presentation, and recorded all three codes, enter them in the boxes below *in the order they appear in the video* and click SUBMIT. If you enter the codes correctly, your information will be submitted to the USF Office of Continuing Professional Education and you will receive your CME credit.

The University of South Florida College of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians. The University of South Florida College of Medicine designates this educational activity for a maximum of one hour in category 1 credit towards the AMA Physicians Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

A certificate for the appropriate number of credits will be mailed to you from the University of South Florida. Please allow 4 to 5 weeks to receive your certificate.

CODE #1 :

CODE #2 :

CODE #3 :

SUBMIT

Equipment and Software Used for Various Aspects of Operation

Acquisition:

The Following equipment is used to acquire video and audio assets for the MCN:

Sony DVCAM DSR 130: broadcast quality video camera used to capture video assets.

The following accessories are used with this camera package:

AC-550 power supply

DXF-41 monochrome viewfinder

VCT-U14 tripod adapter plate

DC-520 Battery case

Anton Bauer propak batteries and accessories

Sony NP batteries and accessories

Seinheiser BFR 1081 wireless microphone kit: used to capture high quality audio needed for web distribution

CSI Daiwa tripod (provides stable platform for camera)

Lowell light kits: used in field acquisition when existing lighting is too low

Digitizing and Posting:

Media 100: Used to edit and prepare programs for the MCN. This process includes compiling raw footage, digitizing, and organizing raw footage into a complete program.

Software includes:

Media 100 xl: use to digitize and edit raw footage

CG Studio: used to add graphics and import graphics from other software programs (i.e. Adobe photoshop, illustrator, etc.)

Boris Effects: used to add special effects and improve the quality of the program being edited

Platform: Macintosh power PC 9600

Hammer Disk Array (for storage)

Sony DSR - 85 DVCAM recorder/player: used to feed video/audio assets into the Media 100.

Sony DSR - 80 DVCAM recorder/player: used to feed video/audio assets into the Dell Workstation.

Sony SVO - 5800 SVHS player/recorder: Used to export video/audio assets for the MCN that were shot on

1/2 VHS and SVHS formats.

Sony RM 450 remote controller: Used to control the DSR-80 and SVO 5800. Can also be used with the DSR 85 and the JVC BR - S822DXU.

JCV BR- S822DXU SVHS editing recorder/player: used to edit and record SVHS footage.

Mackie 8 ch. Audio board: used to optimize audio before digitizing for highest possible quality

DELL Computer H266 MHZ and accessories and software (windows, real producer, Adobe premier, Adobe Photoshop, etc.) used to prep, digitize and export video/audio assets for the MCN. Also used as an office workstation.

Microsoft Word, PowerPoint

Osprey Video Card

Adobe Premiere 5.1, Photoshop 5, Illustrator 8

SoundForge

Macromedia Flash 4, Fireworks 2, Dreamweaver 2

Assembling Assets

Assembling Assets

Summary of Process:

The purpose of this document is to outline the steps necessary to author streaming multimedia presentations for the Web using TAG Composer 2.0. This document assumes basic knowledge of Adobe Photoshop, Macromedia Fireworks, SoundForge and RealProducer Plus G2. You will collect assets and assemble them for a slide show presentation on the web which physicians can watch in order to earn continuing medical education credits. The videographer will tape an actual presentation given here at Moffitt. He then digitizes the video and saves it out as a series of pics. He also digitizes the audio and figures out at what time a slide should show up corresponding to the audio.

Preparing:

1. The videographer will e-mail you the timings.
2. Print out the attached word document, which are the timings.
note: Timings refer to when a slide should appear corresponding to the audio.
3. Save the e-mail message in your personal folder in Outlook.
4. Write down the title of the presentation and the speaker(s) name(s) on the timings print out.
5. You can find the assets following this path: H:\mrc03\mcn\groundrounds\tobedone - the folder should be named with the 6 digit date and the first 4 letters of the speakers last name. If it is not named in this fashion then, after finding out the speakers last name, go ahead and name it correctly. Reason being, sometimes there will be two different presentations on the same day. Inside should either be the slides as .pics or as a PowerPoint presentation.
6. Move the appropriate folder from to **tobedone** folder into the **original assets** folder found in the groundrounds directory.
7. Copy the same folder onto your hard drive into the following directory: C:\Program files\TAG\composer 2.0\media. These are the assets you'll want to work with, in case something goes wrong, you have a backup. *Always* put the assets your working with on your hard drive and leave the original assets on the network drive as backup.

Do your Photoshop Magic!

You will use Photoshop to fix any slides that need to be fixed then resize and rename them. Your original assets will be either 640X480@72dpi pics, 512X384@72dpi .tiffs or a PowerPoint presentation.

If the're pics or .tiffs... then they need to be saved out in Photoshop as 512X384 @ 72dpi jpegs saved out at low quality set at 1.

If the're a PowerPoint presentation... you have to do one more step. You'll have to save the presentation out in PowerPoint as JPEG File Interchange Format. Then fix them in Photoshop and save them out as 512X384 @ 72dpi jpegs saved out at low quality set at 1.

Always fix any slides that are crooked or out of focus in Photoshop. Make them look as good as you can without having to re-type anything. Use your own judgement. Try not to take more than an hour or so fixing slides.

Presentation Codes – for CME or CEU credit

1. Make up three 4-digit codes and put them into the database table called Presentation codes
2. The graphics can be found in the grand rounds folder which should always go in the lower right corner of 3 different slides. One in the beginning, middle, and end.
3. Keep record of which slide # and what time the code will appear on the word document found in the Grand Rounds folder.

Optimize the JPEGs in Fireworks:

1. Open Fireworks
2. Choose **Batch...** from the File Menu.
3. Select the folder the .jpeg's are in (*which should be: C:/Program Files/TAG/Composer 2.0/Media/appropriate folder*) and click ok.
4. Choose Presets - **JPEG Quality 80%**, scale stays at 100% and click ok.
5. It will automatically do it; when it's done, you can close Fireworks.

Audio

1. Open the .rm or .wav file first just by double clicking on it to make sure there is no silence at the end. If so, open it in Soundforge and delete it. **DON'T** delete anything other than silence at the end or you will make all the timings off.
2. **Make a note on the timings sheet the ending time for future use in TAG.**

If the audio is a .wav file

1. Open **RealProducer Plus G2**
2. You will be asked to choose a recording wizard. Choose **Record from File** and click on **Next**.
3. You will then get a Real Wizard window. Click on **Browse...** and find the folder. It should be on your hard drive in the same folder as your jpeg's. Select the .wav file. Click on **Open**. Then click on **Next**.
4. You will get a RealMedia Clip Information window. Make sure the title is correct and the Author is Moffitt Cancer Network and the copyright says 1999 then click **Next**.
5. Choose **Sure Stream** for the File Type then click on **Next**.
6. Check **ALL BOXES** for the Target Audience then click on **Next**.
7. Select **Voice Only** as the audio format then click on **Next**.
8. In the Output file window - just **verify** and click on **Next**. It will default to the correct folder and name it correctly with the proper file extension.
9. Click on **FINISH** after verifying all the information.
10. Then click on **START** - this should take approximately 15-20 minutes.
11. When finished - close RealProducer.

**note: I always delete the .wav file on my C drive because you have a back up on the network in the original assets folder. You will only need the Real Media file in TAG.*

Assembling the assets in TAG:

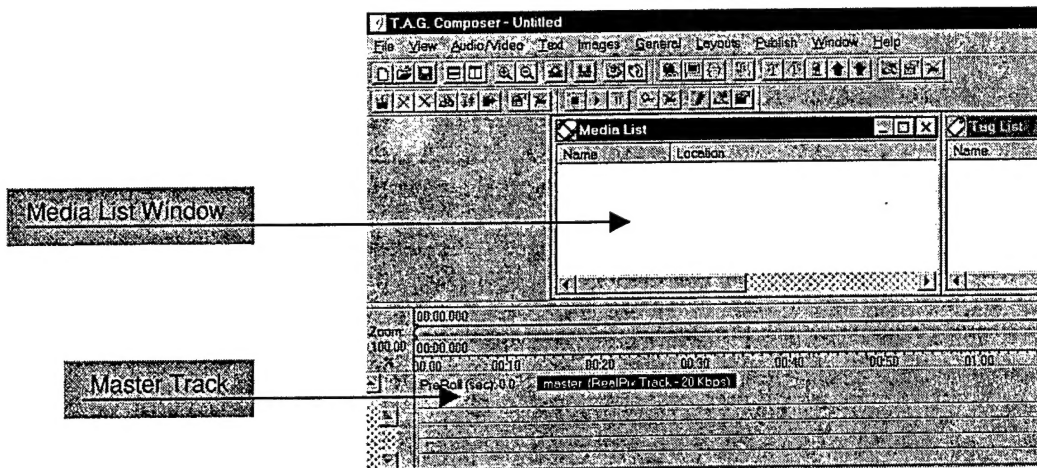
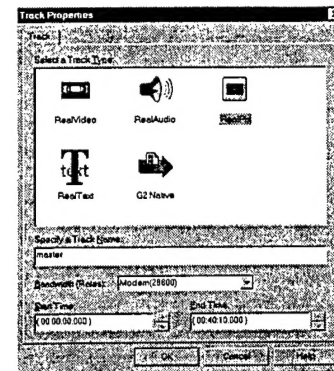
Before you begin to author your presentation you should have the following media assets available:

1. Slide images (fixed, sized and optimized)
2. Audio file in Real Media format.
3. Slide Timings
4. Title of the presentation and the speaker's name and title.
5. The ending time of the audio.

*Just a note about TAG - it crashes all the time. Keep your Resource Meter open so that you can keep an eye on your resources. Make sure that TAG and the resource meter are the only programs running.

Getting Started with TAG Composer 2.0

1. Open TAG Composer 2.0.
2. Choose New from the File menu.
3. You will get a window like the one to the right.
 - a. Choose Real Pix
 - b. Leave Track name "master"
 - c. Change the bandwidth to Modem(28800)
 - d. Specify the End Time. This should be the same as the end time from the audio you noted earlier on the timing sheet.
 - e. Click OK
4. Right click on anywhere in the Master Track and **turn off Show Thumbnails**.

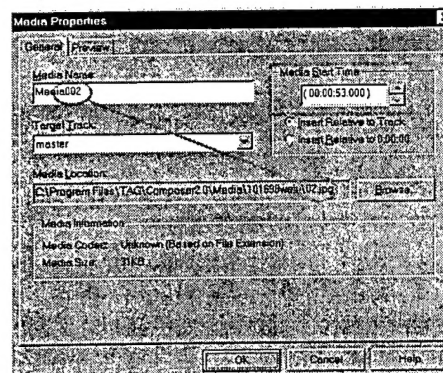


Adding the Media (jpegs – slides)

This is the part when your resources will go down. After adding about 30 slides you will need to save the project and close TAG. If your resources go all the way back up into the green then open TAG and start again. If your resources don't go back up then restart your computer.

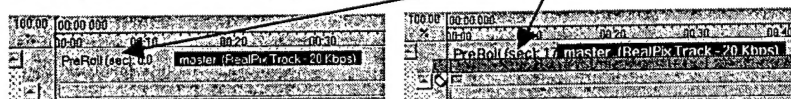
Save the Project once the meter is in the yellow! It will default to the correct folder (C:/Program Files/TAG/Composer 2.0/Projects). Name it correctly – 6digit date and first four letters of the speakers last name.

1. Right click anywhere in the Media List Window and choose **add media**.
2. **Find** the first slide (which should be: C:/Program Files/TAG/Composer 2.0/ Media/appropriate folder) and click OK.
3. You will then get a window like this one. Only with the first slide will you not have to specify a start time because it will default to 0. However, for all others, **refer to your timing sheet and input the correct Media Start Time**.
4. Make sure the Media name and the jpeg are the same number.
5. Click OK.
6. **Repeat steps 1-6** until all the jpegs have been added.



Don't forget to keep an eye on your resource meter!!!

Note: the procedure of adding media always makes TAG crash so **save** when the resource meter is in the yellow. You will know when the program has crashed when the letters of the interface get bold. If this happens, don't save and close TAG, RESTART and pray that it did not corrupt your file.



Additional Information on RealPix Tracks

There are several factors you will want to take into account when creating **RealPix tracks** in your streaming media presentation. The file size of your slide images should be kept **under 20k** if possible. The track's **Pre Roll** value should be **less than 20 seconds** if possible. Pre Roll represents the time it takes (in seconds) to download and cache images in a streaming media presentation. This cache keeps the presentation playing even if Internet bandwidth is reduced.

Adding the Audio

1. Select the **Audio/Video** pull down from the menu bar. Select **Add Real Audio Track**. A blank audio track will be added to your project window.
2. Right click on the new track and choose **Track Properties...**
3. Specify the correct **End Time**, which is the same as the end time you noted earlier on the timings sheet.
4. Right click anywhere in the Audio Track and select **Add Media**.
5. Choose the Real Media audio file which should be in the same folder as the jpegs.
6. Click OK.

By this point you should have the following things in your projects:

1. Slide images placed on the Timeline in sequence
2. An audio file

You want to check to make sure:

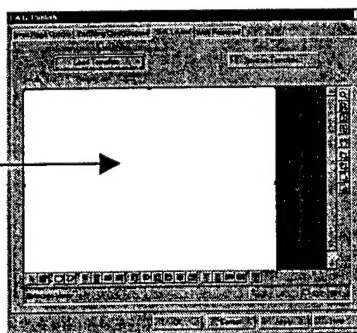
1. Pre Roll is 20 seconds or less
2. No overlapping slides

SAVE THE PROJECT!

Publishing the Project

1. Select the **Layout** pull down from the menu bar then choose **Select SMIL Layout**.
2. Click on **Load Template** in the window that appears then choose **MCN Presentation** from the list. Click OK.
3. Right click on the white region of the window and select **Players Properties**.
 - a. Click on the **SMIL Sound Track List** tab, make sure the name of your audio track is selected.
 - b. Click on the **General Information** tab and fill in the appropriate information. The title of the Presentation, Copyright should be H. Lee Moffitt Cancer Center & Research Institute, and the author should be the speakers name.
 - c. Click OK.
4. Right click in the white region of the window again and select the **Region Properties**.
 - a. Click on the Region Track List tab and make sure that the name of your Real Pix track is selected *(should be master unless you renamed it)*.
 - b. Click OK.
5. Click OK again to close the TAG Layout window.

White region of the window



6. **Save the Project!**
7. Click on the Publish pull down menu and select **Publish**. Let it do its thing and you're done with TAG. (whew!)

Where did it go and what do I do with it?

When you published the project you actually told TAG to create a SMIL (streaming media interface language) file. This is the actual file that people will see on the web.

This is where the SMIL file can be found:

C:/Program files/TAG/composer 2.0/projects/name of project folder.

1. Open the folder.
2. Rename the realSystemG2 file (which is the actual SMIL file) to the name of the project.
- You should now have a master folder with all the jpegs in it and a realaudio01 folder with the audio file in it and the SMIL file properly named. The SMIL file cannot work properly unless these two folders stay with it at all times. When I say move or copy the SMIL file I'm referring to the whole folder which should be named the same as the project - *6 digit date and the first four letters of the speakers last name.*

Backup

1. Make a back up onto your **personal network drive** of the TAG project and the SMIL folder.
2. Put the SMIL folder *not* the TAG project into the following folder:
Merc on HLMRC03/mcn/groundrounds/done.

Put it in it's place

Put the SMIL file in the Courses folder which can be found using the following path:
HLMRC02/content/courses

Create a RAM file

A RAM file is a pointer to the SMIL file

1. Open Notepad
2. Type **rtsp://video.moffitt.usf.edu/courses/name of folder/name of smil file.smi**
Example: **rtsp://video.moffitt.usf.edu/courses/101698wein/101698wein.smi**
3. Save as: **smil file name.ram** (example: **101698wein.ram**) make sure you change the file format to **all files** or you will have saved a text document instead of a ram file.
Save notepad document into **HLMRC04/webprojects/mcn/rams.**

You can now delete the media, the project and SMIL folder off your Hard Drive.

Final Stage: Database

Open the Database and check to see if the presentation is already there. The last thing to do is enter the Presentation Location, Length, any other information you have which is not completed. Then click on the Add Search Terms button. On the next page is a screen shot of the database with the proper sections hi-lited.

You're Done!!

Database Location: Web_projects on **Hlmrc04/Mcn/database/presentations**